

Alexandrina L. Dumitrescu

Chemicals in Surgical Periodontal Therapy



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Preface

“...Nature doesn’t make useless things!”
Aristotele, 384–322 a.C.

Periodontitis is an inflammatory disease characterized by the destruction of periodontal ligament, root cementum and alveolar bone as a tissue response to microbial plaque accumulation on the tooth root surface.

For periodontal therapy that aims at the regeneration of the periodontal tissues, i.e., the restoration of their initial form, architecture and function, many choices of therapeutic procedures must be weighed before the proper one is chosen. Periodontal treatment includes conventional methods such as scaling and root planing, periodontal surgery with or without osseous surgery and new approaches such as guided tissue regeneration, root conditioning agents, the use of different grafting materials, enamel matrix derivative and their combination. In recent years, advances in molecular and cellular biology led to the study of growth factors’ potential role in promoting periodontal regeneration and their use as an alternative therapeutic approach.

The examination of the patient, characteristics of the defect and knowledge of materials used in surgical periodontal therapy are all factors that must be entertained before the surgery begins. There are many options in periodontal regenerative surgery materials from which to choose. Knowledge of all of their advantages and disadvantages would allow a dentist to obtain the maximum of their benefits in the best interest of their patients.

Alexandrina L. Dumitrescu

Contents

1 Guided Tissue Regeneration Barriers	1
1.1 Biologic Basis of Guided Tissue Regeneration (GTR).....	1
1.2 Summary of Studies Evaluating the Efficacy of GTR in the Treatment of Infrabony Defects	1
1.2.1 Histological Studies	2
1.2.2 Clinical Studies	2
1.3 Summary of Studies Evaluating the Efficacy of GTR in the Treatment of Furcation Lesions.....	7
1.3.1 Histological Studies	9
1.3.2 Clinical Studies	9
1.4 Summary of Studies Evaluating the Efficacy of GTR in the Treatment of Gingival Recession	14
1.4.1 Histological Studies	14
1.4.2 Clinical Studies	14
1.5 Adjunctive Use of Antibiotics in GTR Treatment.....	22
1.6 Advantages and Disadvantages of the Use of GTR Treatment	23
1.7 GTR Barriers for Periodontal Regeneration	24
1.7.1 The Qualities of an “Optimal GTR Barrier” for Periodontal Regeneration	24
1.7.2 Nonresorbable Membranes	25
1.7.3 Bioresorbable Membranes	31
1.7.4 New Trends in Guided Tissue Regeneration Barriers Development	52
References	58
2 Bone Grafts and Bone Graft Substitutes in Periodontal Therapy.....	73
2.1 Autogenous Grafts	73
2.1.1 Intraoral Autografts.....	74
2.1.2 Extraoral Autografts.....	75
2.2 Allografts	77
2.2.1 Freeze-Dried Bone Allografts (FDBA).....	81
2.2.2 Demineralized Freeze-Dried Bone Allografts (DFDBA)	81
2.3 Xenografts.....	85
2.3.1 Anorganic Bovine-Derived Bone Xenograft (BDX)	86
2.3.2 Anorganic Porcine-Derived Bone Xenograft.....	90
2.3.3 Coralline Calcium Carbonate.....	92

2.4	Alloplasts (Alloplastic Synthetic Grafts).....	94
2.4.1	Polymethylmethacrylate and Polyhydroxyethylmethacrylate (PMMA-PHEMA) Polymers	94
2.4.2	Demineralized Dentin Matrix (DDM)	95
2.4.3	Hydroxylapatite (HA)	95
2.4.4	Calcium Phosphate Cement (CPC).....	103
2.4.5	β -Tricalcium Phosphate (TCP)	107
2.4.6	Calcium Sulfate.....	114
2.4.7	Bioactive Glasses (BG).....	116
2.4.8	Oily CaOH_2 Suspension.....	121
2.4.9	Porous Titanium Granules	123
2.5	Composite Grafts	123
2.6	Factors Impacting Treatment Outcome	125
2.6.1	Criteria for Evaluation of Graft Success for Periodontal Regeneration	125
2.6.2	Factors Influencing Graft Success.....	126
	References	127
3	Enamel Matrix Derivative for Periodontal Tissue Regeneration.....	145
3.1	EMD Formulation.....	145
3.2	Clinical Safety of EMD	146
3.3	Biomimicry	146
3.4	Mechanisms Underlying the Supportive Effects of EMD	146
3.4.1	In Vitro and In Vivo Experiments	146
3.4.2	Animal Experiments	147
3.4.3	Human Histological Studies	147
3.5	Summary of Studies Evaluating the Efficacy of Emdogain in the Treatment of Infrabony Pockets (Vertical Bone Loss)	171
3.5.1	Nonsurgical Periodontal Therapy	171
3.5.2	Surgical Periodontal Therapy.....	171
3.5.3	Hard Tissue Response After Emdogain Treatment of Intrabony Pockets	189
3.5.4	Factors That Determine Emdogain Outcomes in the Treatment of Infrabony Defects	192
3.6	Summary of Studies Evaluating the Efficacy of Emdogain in the Treatment of Furcation Lesions.....	195
3.7	Summary of Studies Evaluating the Efficacy of Emdogain in the Treatment of Supra-alveolar-type Defects.....	197
3.8	Summary of Studies Evaluating the Efficacy of EMD in the Treatment of Gingival Recession.....	198
3.9	Clinical Studies Evaluating the Effect of EMD on Early Wound Healing	208
3.10	Clinical Studies Evaluating the Effect of EMD on Periodontal Healing of Replanted Teeth	208
3.11	Advantages of the Use of Emdogain Gel.....	209
	References	209

4 Chemical Root Surface Modifiers in the Treatment of Periodontal Disease	217
4.1 Citric Acid	217
4.2 Tetracycline HCl.....	220
4.3 EDTA	221
References	224
5 The Use of Biologic Mediators for Periodontal Regeneration.....	227
5.1 Growth Factor Delivery for Oral and Periodontal Tissue Engineering	227
5.1.1 Biomaterials for Growth Factor Delivery	230
5.1.2 Configurations of Growth Factor Delivery Carriers	234
5.2 The Use of Platelet-Rich Plasma (PRP) for Periodontal Regeneration	235
5.2.1 Preparation of Platelet-Rich Plasma	238
5.2.2 Handling and Application of Platelet-Rich Plasma	239
5.2.3 Safety	241
5.2.4 Human Studies on Platelet-Rich Plasma.....	241
5.2.5 Potential Advantages and Limitations of PRP	243
5.3 Human Platelet-Derived Growth Factor-BB (PDGF).....	243
5.4 Peptide P-15.....	246
5.5 Insulin-Like Growth Factors.....	249
5.6 Fibroblast Growth Factor-2.....	250
5.7 Transforming Growth Factor- β	254
5.8 Bone Morphogenetic Proteins	262
5.9 Growth Factor Combinations.....	292
References	293
Index.....	305

Guided tissue regeneration (GTR) typically refers to regeneration of periodontal attachment. Regeneration refers to the reproduction or reconstitution of a lost or injured part, in contrast to *repair*, which describes healing of a wound by tissue that does not fully restore the architecture or the function of the part. *Periodontal regeneration* is defined histologically as regeneration of the tooth's supporting tissues, including alveolar bone, periodontal ligament, and cementum over a previously diseased root surface. *New attachment* is defined as the union of connective tissue or epithelium with a root surface that has been deprived of its original attachment apparatus. This new attachment may be epithelial adhesion and/or connective tissue adaptation or attachment and may include new cementum. It is to be distinguished from *reattachment*, which describes the reunion of epithelial and connective tissue with a root surface (American Academy of Periodontology 2001; Wang et al. 2005).

1.1 Biologic Basis of Guided Tissue Regeneration (GTR)

Successful periodontal regeneration relies on the re-formation of an epithelial seal, deposition of new acellular extrinsic fiber cementum and insertion of functionally oriented connective tissue fibers into the root surface, and restoration of alveolar bone height (Villar and Cochran 2010). Following flap elevation, the instrumented root surface can be repopulated by epithelial cells, gingival connective tissue cells, bone cells and periodontal ligament cells. Under normal healing conditions, epithelial cells rapidly migrate in an apical direction to reach the most apical portion of the instrumentation, forming a long junctional epithelium

and preventing the formation of a new attachment. The barrier membrane prevents gingival epithelium and connective tissue expansion and allows cells from the periodontal ligament and bone to repopulate the root surface and to form a new periodontal attachment (Cortellini and Tonetti 2000; Nyman et al. 1980; Karring et al. 1980, 1985; Bosshardt and Sculean 2009) (Figs. 1.1 and 1.2). Besides favoring selective repopulation of the wound area, physical barriers are also thought to provide protection to the blood clot during the early phases of healing and to ensure space maintenance for ingrowth of a new periodontal apparatus. GTR membranes, as physical barriers, however, provide no biologic effects on the differentiation and proliferation of mesenchymal and periodontal ligament cells, which is likely to limit their clinical efficacy (Villar and Cochran 2010).

1.2 Summary of Studies Evaluating the Efficacy of GTR in the Treatment of Infrabony Defects

According to the classification by Goldman and Cohen (1958), suprabony defects are those where the base of the pocket is located coronal to the alveolar crest. Infrabony defects, on the other hand, are defined by the apical location of the base of the pocket with respect to the residual alveolar crest (Papapanou and Tonetti 2000). With regard to infrabony defects, two types of defects can be recognized: intrabony defects and craters. Intrabony defects are bony defects whose infrabony component affects primarily one tooth, while in craters the defect affects two adjacent root surfaces to a similar extent. Intrabony defects have been classified according to their morphology in terms

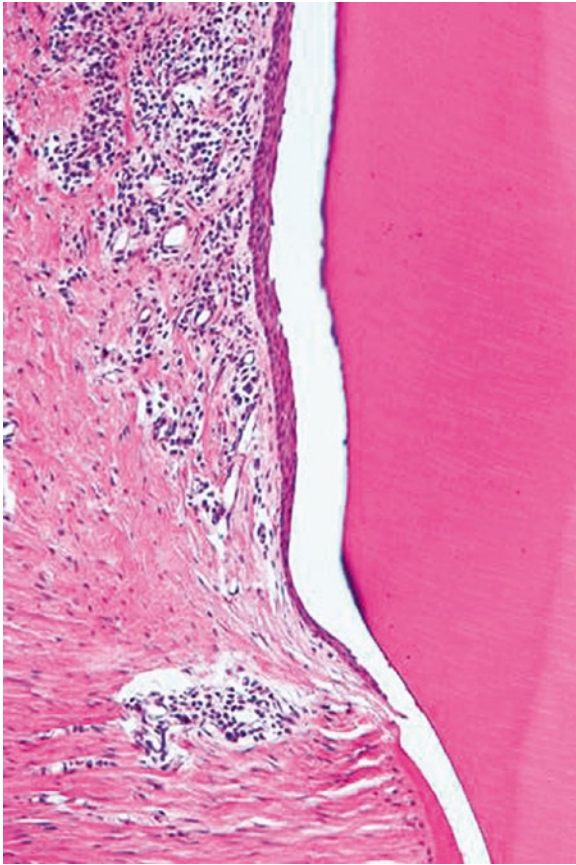


Fig. 1.1 Light micrograph illustrating formation of a long junctional epithelium (*LJE*) ending at the coronal-most end of regenerated cementum (*C*). *D* dentin. (Paraffin section stained with hematoxylin and eosin.) (Bosshardt and Sculean 2009. Reprinted with permission from John Wiley & Sons)

of residual bony walls, width of the defect (or radiographic angle), and in terms of their topographic extension around the tooth. Three-wall, two-wall and one-wall defects have been defined on the basis of the number of residual alveolar bone walls. This represents the primary classification system. Frequently, intrabony defects present a complex anatomy consisting of a three-wall component in the most apical portion of the defect, and two- and/or one-wall components in the more superficial portions. Such defects are frequently referred to as combination defects. Hemiseptal defects, that is, vertical defects in the presence of adjacent roots and where half of a septum remains on one tooth, represent a special case of one-wall defects (Glossary of Periodontal Terms 1993; Papapanou and Tonetti 2000).

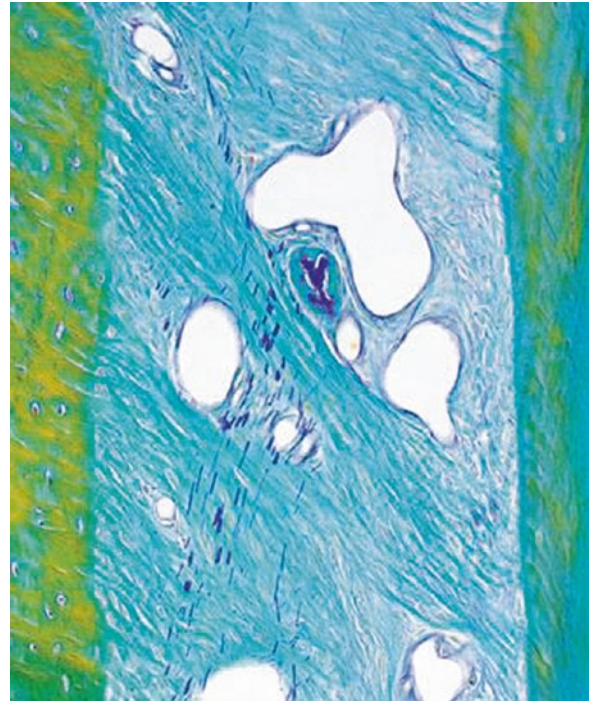


Fig. 1.2 Light micrograph illustrating true periodontal regeneration as demonstrated by new periodontal ligament fibers (*NPLF*) inserting into new bone (*NB*) and new cementum (*NC*). (Paraffin section, oxone-aldehyde-fuchsin-Halmi stain.) (Bosshardt and Sculean 2009. Reprinted with permission from John Wiley & Sons)

1.2.1 Histological Studies

Histological evaluation, however, remains the only reliable method of determining the nature of the attachment apparatus resulting from regenerative procedures. Several studies in animals (Aukhil et al. 1983, 1986; Caffesse et al. 1988, 1994; Caton et al. 1992; Gottlow et al. 1984, 1994; Nyman et al. 1982a; Elharar et al. 1998; Batista et al. 1999; Blumenthal et al. 2003) and some human biopsy material (Becker et al. 1987; Cortellini et al. 1993a, b; Gottlow et al. 1986; Nyman et al. 1982b; Stahl and Froum 1991; Stahl et al. 1990; Laurell et al. 2006) have documented that guided tissue regeneration is capable of promoting new attachment formation.

1.2.2 Clinical Studies

The reported outcomes indicate that the application of nonresorbable or bioresorbable barrier membranes

consistently and predictably results in clinical improvements in intrabony defects (Cortellini and Tonetti 2000).

1.2.2.1 Therapeutic End-Points of Success

The primary outcomes and desirable clinical results of the regenerative treatment of intrabony defects are (1) gain of clinical attachment and bone, (2) fill of the intrabony component of the defect, (3) reduction of pocket depth and (4) minimal gingival recession (Cortellini and Tonetti 2000). The main methods used for evaluation include histology, direct measurement of bone, periodontal probing and radiographic analysis (Reddy and Jeffcoat 1999).

1.2.2.2 Comparison GTR Alone Versus Control/ Placebo/Open Flap Debridement

Several systematic reviews and meta-analyses have reported greater benefits to GTR than open-flap debridement in the treatment of intrabony defects (Laurell et al. 1998; Cortellini and Tonetti 2000; Needleman et al. 2001, 2005; Murphy and Gunsolley 2003; Aichelmann-Reidy and Reynolds 2008).

Laurell et al. (1998) reviewed studies presented during the last 20 years on the surgical treatment of intrabony defects. GTR resulted in significant pocket reduction, a clinical attachment level (CAL) gain of 4.2 mm, and bone fill averaging 3.2 mm.

The weighted mean of the evidence reported in the 11 studies reviewed by Cortellini and Tonetti (2000) indicated that the gain of clinical attachment in sites treated with guided tissue regeneration was 3.4 ± 1.8 mm (95% CI: 3.0–3.7 mm), while the access flap resulted in a mean gain of 1.8 ± 1.4 mm (95% CI: 1.5–2.1 mm). The analysis of the reported clinical outcomes strongly suggests an added benefit derived from the placement of barrier membranes after elevation of an access flap. The frequency distribution of clinical attachment level changes at 1 year has been evaluated by subdividing the data into five classes of probing attachment level changes: loss of attachment, gain of 0–1 mm, gain of 2–3 mm, gain of 4–5 mm and gain of 6 mm or more. Only 2.7% of 651 treated cases lost attachment, while gains of less than 2 mm were observed in 11% of the cases. Most of the sites gained

considerable attachment. In fact, gains of 2–3 mm were observed in 24.8% of the cases, gains of 4–5 mm in 41.3%, and gains of 6 mm or more in 21.2% of defects. These encouraging data demonstrate that guided tissue regeneration is not only efficacious, but also predictable. Regarding changes in bone levels, bone gains ranged from 1.1 to 4.3 mm and seemed to correlate well with the gains in clinical attachment. Reduction of pocket depths is one of the critical end-points of most periodontal procedures, including guided tissue regeneration. An important parameter to evaluate the successful outcomes of guided tissue regeneration, therefore, is the depth of the residual pockets. At 1 year, the weighted mean of residual pocket depths was 3.3 ± 1.2 mm, with a 95% confidence interval ranging from 3.2 to 3.5 mm (Cortellini and Tonetti 2000).

Needleman et al. (2001) evaluated 23 RCT of at least 12 months duration comparing GTR (with or without graft materials) with open flap debridement for the treatment of periodontal infrabony defects up to October 2000. Furcation involvements and studies specifically treating early-onset diseases were excluded. For attachment level change, the weighted mean difference between GTR alone and open flap debridement was 1.11 mm (95% CI: 0.63–1.59, chi-square for heterogeneity 31.4 (df=9), $P < 0.001$), and for GTR+bone substitutes was 1.25 mm (95% CI: 0.89–1.61, chi-square for heterogeneity 0.01 (df=1), $P = 0.91$). Probing depth reduction demonstrated a small but statistically significant benefit for GTR, weighted mean difference 0.80 mm (95% CI: 0.14, 1.46, chi-square for heterogeneity 10.0 (df=4), $P = 0.04$) or GTR+bone substitutes, weighted mean difference 1.24 mm (95% CI: 0.89, 1.59, chi-square for heterogeneity 0.03 (df=1), $P = 0.85$). No significant difference was noted for gingival recession between GTR and open flap debridement. Regarding hard tissue probing at surgical re-entry, a statistically significant greater gain was found for GTR compared with open flap debridement.

Murphy and Gunsolley (2003) assessed the efficacy of guided tissue regeneration (GTR) procedures in patients with periodontal osseous defects compared with surgical controls on clinical, radiographic, adverse, and patient-centered outcomes. GTR procedures resulted in a greater gain in clinical attachment level (CAL) when compared to OFD controls. The difference in gain of CAL between the test barrier types – collagen,

polymeric barriers, ePTFE and an OFD control – was 0.95 ± 0.47 , 0.92 ± 0.18 and 1.61 ± 0.25 mm, respectively. Similar findings were demonstrated with PD reduction for all barrier types ($P < 0.0001$). The difference in PD reduction between the test barrier types – collagen, polymeric barriers, ePTFE and an OFD control – was 1.06 ± 0.37 , 0.89 ± 0.14 and 1.41 ± 0.20 mm, respectively (Murphy and Gunsolley 2003).

In the meta-analysis performed by Needleman et al. (2005), 16 studies presented attachment level data for GTR alone: 8 parallel group trials and 8 split-mouth studies (Table 1.1). The results for this analysis show a statistically significantly greater attachment gain for test groups compared with open flap debridement. For GTR, the mean difference between test and control was 1.22 mm (95%CI random effects [0.80,1.64], chi-square for heterogeneity 69.1 (df=15), $P < 0.001$, $I^2 = 78\%$). A significantly greater probing depth reduction for GTR was demonstrated, weighted mean difference 1.21 mm (95%CI [0.53,1.88], chi-square for heterogeneity 62.9 (df=10), $P < 0.001$, $I^2 = 84\%$). Regarding gingival recession change, a statistically significant difference between GTR and open flap debridement controls was evident (mean difference 0.26 mm, 95%CI random effects [0.08,0.3], chi-square for heterogeneity 2.7 (df=8), $P = 0.95$), with a greater change in recession from baseline for the control group. For GTR, a statistically significant greater gain in hard tissue probing was found for GTR compared with open flap debridement. This amounted to a weighted mean difference of 1.39 mm (95%CI [1.08,1.71], chi-square for heterogeneity 0.85 (df=2), $P = 0.65$) (Needleman et al. 2005).

Aichelmann-Reidy and Reynolds (2008) examined the relative variability in clinical outcome measures, independent of the magnitude of gains, in regenerative studies comparing GTR to OFD therapy for the management of intrabony defects. GTR therapy was associated with significantly greater gains in CAL (4.37 ± 1.32 vs. 3.26 ± 0.87 , respectively) and reductions in PPD (3.21 ± 1.36 vs. 2.06 ± 1.18 , respectively) than OFD alone. Mean gains in defect fill were nonsignificantly greater following GTR therapy than OFD alone (1.95 ± 0.71 vs. 1.38 ± 0.82 , respectively). GTR therapy also was associated with a significantly lower mean percent of coefficient of variability for CAL gain compared to OFD alone (50.6 ± 5.0 vs. 68.7 ± 8.2 , respectively); however, variability in PPD reduction (37.3 ± 3.3 vs. 42.5 ± 4.9) and defect fill (101.5 ± 36.6)

vs. 99.4 ± 36.6) was similar following treatment with GTR and OFD, respectively (Aichelmann-Reidy and Reynolds 2008).

Differences in search strategy, inclusion of both randomized and nonrandomized studies, and of studies of shorter duration of follow-up may have accounted for the differences recorded in these systematic reviews and meta-analyses (Needleman et al. 2005).

1.2.2.3 Comparative Studies Between Treatment of Infrabony Defects with Non-bioresorbable and Bioresorbable Materials

The reported outcomes indicate that the application of nonresorbable or bioresorbable barrier membranes consistently and predictably results in clinical improvements in intrabony defects. Clinical improvements associated with GTR were independent of the type of barrier membrane used, i.e., nonresorbable or resorbable (Villar and Cochran 2010). Guided tissue regeneration treatment of 351 defects (20 studies) with nonresorbable barrier membranes resulted in clinical attachment level gains of 3.7 ± 1.8 mm; this was similar to the results obtained treating 592 intrabony defects (17 studies) with bioresorbable barrier membranes (3.6 ± 1.5 mm) (Cortellini and Tonetti 2000). Meta-analysis of Murphy and Gunsolley (2003) also failed to demonstrate a significant difference between ePTFE and the polymeric barriers ($P > 0.05$). In contrast, Parrish et al. (2009) reported significant differences in CAL gains between non-bioabsorbable membranes without grafts (3.77 mm) versus collagen membranes (2.36 mm).

1.2.2.4 The Results of Comparative Studies Between Guided Tissue Regeneration Treatment of Infrabony Defects with or without the Adjunctive Use of Other Regenerative Techniques

Allogeneic and alloplastic bone substitutes have been implanted to support guided tissue regeneration membranes and to “enhance” periodontal regeneration (Becker and Becker 1999).

As summarized by Sculean et al. (2008), in animal models combined therapy (i.e., graft + GTR) resulted in clinically and histologically superior results

Table 1.1 Summary of meta-analyses for different outcomes and sensitivity analyses for different groups of studies

Outcome	Study type	No. of studies	Heterogeneity chi-square statistic	Heterogeneity P-value	Mean difference	95%CI (random effects)
Attachment gain	GTR only	16	69.1	<0.001	1.22	[0.80, 1.64]
Attachment gain	GTR parallel groups	8	34.4	<0.001	1.71	[1.02, 2.40]
Attachment gain	GTR split-mouth	8	18.5	0.01	0.79	[0.37, 1.21]
Attachment gain	GTR + bone substitutes	2	0.01	0.91	1.25	[0.89, 1.61]
Attachment gain	Sensitivity analysis: GTR only assuming intrapatients correlation = 0	16	63.3	<0.001	1.24	[0.82, 1.66]
Attachment gain	Sensitivity analysis: GTR only with adequate allocation concealment	7	30.4	<0.001	1.63	[0.85, 2.42]
Attachment gain	Sensitivity analysis: GTR only, examiner blinded	8	44.8	<0.001	1.27	[0.50, 2.04]
Attachment gain	Sensitivity analysis: GTR only, therapist blinded	8	32.4	<0.001	1.19	[0.57, 1.81]
Attachment gain	Sensitivity analysis: GTR only, examiner and therapist blinded	3	4.4	0.11	0.41	[-0.33, 1.08]
Pocket depth reduction	GTR only	11	62.9	<0.001	1.21	[0.53, 1.88]
Pocket depth reduction	GTR parallel groups	5	43.6	<0.001	1.59	[0.21, 2.97]
Pocket depth reduction	GTR split-mouth	6	9.1	0.11	0.87	[0.38, 1.36]
Pocket depth reduction	GTR + bone substitutes	2	0.03	0.85	1.24	[0.89, 1.59]
Gingival recession	GTR only	8	2.6	0.92	0.26	[0.08, 0.44]
Gingival recession	GTR parallel groups	4	0.97	0.81	0.15	[-0.12, 0.42]
Gingival recession	GTR split-mouth	4	0.41	0.94	0.35	[0.11, 0.60]
Gingival recession	GTR + bone substitutes	1	N/A	N/A	-0.33	[-0.43, 0.23]
Bone gain.	Surgical re-entry GTR only	3	0.85	0.65	1.39	[1.08, 1.71]
Bone gain	Surgical re-entry GTR + bone substitutes	1	N/A	N/A	3.37	[3.14, 3.61]

Source: Needleman et al. (2005). Reprinted with permission from John Wiley & Sons
 GTR guided tissue regeneration

compared with the single therapies (Kim et al. 1998b; Blumenthal et al. 2003).

Meta-analysis of Murphy and Gunsolley (2003) did not reveal any difference in CAL gain between test (barrier in addition to a particulate graft material) and control (barrier alone) groups.

Needelman et al. (2005) evaluated only two studies for GTR+bone substitutes (Blumenthal and Steinberg 1990; Kim et al. 1998a) and the mean difference for these two studies was 1.25 mm (95%CI [0.89,1.61], chi-square for heterogeneity 0.01 (df=1), $P=0.91$), similar to the overall result for GTR alone. The results demonstrated a significantly greater probing depth reduction for GTR+bone substitutes with a weighted mean difference of 1.24 mm (95%CI: [0.89,1.59], chi-square for heterogeneity 0.03 (df=1), $P=0.85$) similar to that for the GTR alone. Slightly greater recession for test than controls, with a mean difference of -0.33 mm (95%CI $[-0.43, 0.23]$) was showed. Regarding gain in hard tissue probing, for GTR+bone substitutes the difference was statistically significant, with a mean difference of 3.37 mm (95%CI [3.14, 3.61]) (Needleman et al. 2005).

Parrish et al. (2009) reported significant differences in CAL gains between collagen membranes with grafts (3.50 mm) versus open flap debridement with or without grafts (1.98 vs. 1.77 mm). Analysis of research using EMD with graft material and/or membranes resulted in an average mean CAL gain of 3.89 mm, with a range of 3.00–5.8 mm.

Villar and Cochran (2010) suggested that incorporation of bone grafts enhances clinical attachment and vertical bone gain in one-wall intrabony defects treated with barrier membranes, while regeneration outcomes obtained from GTR treatment of two-walled and combined one-, two-, and three-walled intrabony defects are not enhanced by the addition of grafting materials. The authors hypothesized that incorporation of bone grafts only enhances clinical results in non-self-supporting defects where the incorporation of grafting materials may prevent the collapse of the barrier.

1.2.2.5 Efficacy of Root-Conditioning Agents in Conjunction with Guided Tissue Regeneration in the Treatment of Infrabony Defects

Various root biomodification regimes have been advocated as adjuncts to regenerative procedures. Citric

acid and tetracyclines have been used to remove the smear layer from instrumented root surfaces (Becker and Becker 1999). There is no evidence that such root biomodification enhances periodontal regeneration in humans (Mariotti 2003; Handelsman et al. 1991; Kersten et al. 1992).

1.2.2.6 Long-Term Evaluation

Several studies revealed that the results of GTR are stable over long periods of time (Nickles et al. 2009; Pretzl et al. 2008; Eickholz et al. 2007, 2004b; Kim et al. 2002; Stavropoulos and Karring 2004).

1.2.2.7 Factors Affecting the Outcomes Achieved with Guided Tissue Regeneration in the Treatment of Infrabony Defects

Several studies have evaluated three types of possible sources of variability of the clinical outcomes of guided tissue regeneration: (1) the patient, (2) the morphology of the defect and (3) the guided tissue regeneration procedure and the healing period (Cortellini and Tonetti 2000). Kornman and Robertson (2000) classified factors that may influence the successful management of periodontal osseous defects. Their classification includes bacterial contamination, innate wound-healing potential, local site characteristics and surgical procedure/technique.

Along these lines, Cortellini and Tonetti (2000) suggested operative decision trees (Figs. 1.3 and 1.4) based on a stepwise approach with subsequent decision nodes, to assist clinicians in the process of selecting the proper treatment strategy in different clinical cases. The starting point of the decision process is the selection of the patient. According to the evidence, patients with less than 15% of sites presenting with plaque and residual infection, nonsmokers with a high degree of compliance, and the systemically healthy are the best candidates for guided tissue regeneration. The second step is the selection of the defect. Defects presenting with a radiographic angle of 25° or less, an intrabony component deeper than 3 mm and gingival tissues at least 1 mm thick have the greatest chances to result in consistent amounts of clinical attachment and bone gains, irrespective of the number of residual bony walls. The thickness of the gingival tissues, if

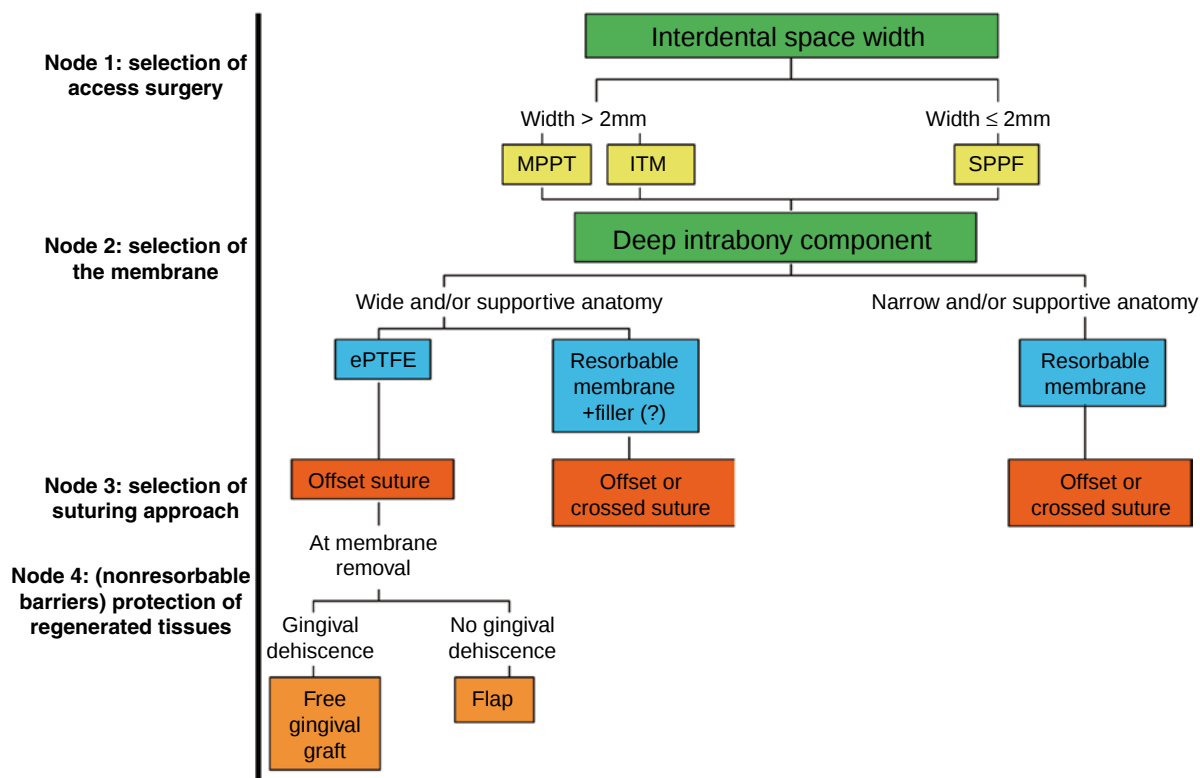


Fig. 1.3 Decision tree: non-esthetically sensitive sites. The objective of treatment is to increase the periodontal support and decrease the probing pocket depth. *MPPT* modified papilla preservation technique, *ITM* interproximal tissue maintenance,

SPPF simplified papilla preservation flap, *ePTFE* expanded polytetrafluoroethylene (Cortellini and Tonetti 2000. Reprinted with permission from John Wiley & Sons)

unfavorable, can be improved with mucogingival surgery (Cortellini and Tonetti 2000).

Wang et al. (2005) categorized factors that may limit regenerative healing after GTR surgery into barrier-independent (e.g., poor plaque control, smoking, occlusal trauma, suboptimal tissue health, mechanical habits that interfere with healing, inadequate overlying keratinized tissue and tissue thickness, improper surgical technique, premature plaque colonization and early mechanical insult, and loss of wound stability) and barrier-dependent (e.g., inadequate root-barrier seal, nonsterile technique, instability of the membrane, and premature membrane exposure/loss) (Wang and MacNeil 1998). Most important among these are presence of a smoking habit, poor plaque control and premature exposure of the barrier (Wang et al. 2005).

Factors affecting the outcomes achieved with guided tissue regeneration in the treatment of intrabony lesions are summarized in Table 1.2.

1.3 Summary of Studies Evaluating the Efficacy of GTR in the Treatment of Furcation Lesions

In the course of periodontal history, several techniques have been proposed and promoted to treat furcated molars and thus improve their prognosis. Grade I furcations have generally been well managed with routine periodontal surgical procedures aimed at thoroughly debriding the lesion, reduce pockets and expose the furcation entrance for adequate plaque control. Grade III furcations have required more extensive resective therapy such as tunneling, root amputation or hemisection with the aim of eliminating the lesion and thus also allowing for proper infection control. Grade II furcations have presented, however, a clinical problem that has troubled clinicians for many years. Various regenerative procedures have been tried with the aim of closing grade II furcations, such as open flap

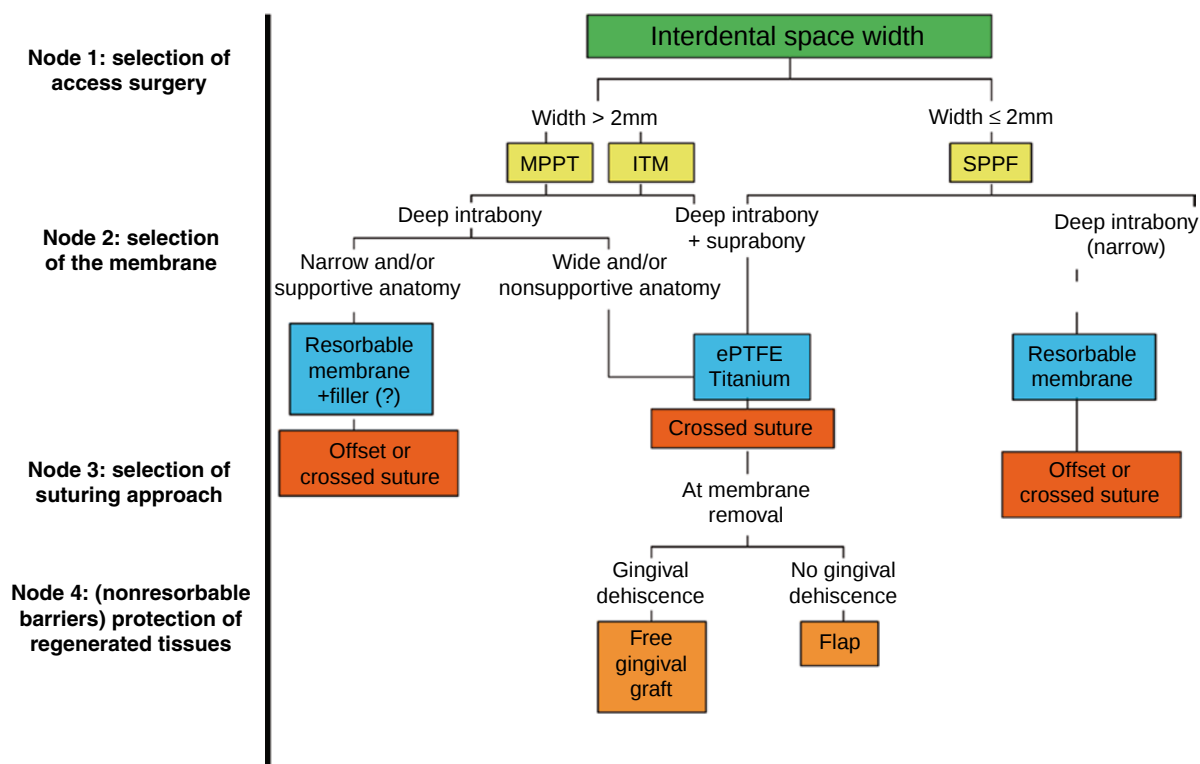


Fig. 1.4 Decision tree: esthetically sensitive sites. The objective of treatment is to completely resolve the defect and minimize recession. *MPPT* modified papilla preservation technique, *ITM*

interproximal tissue maintenance, *SPPF* simplified papilla preservation flap, *ePTFE* expanded polytetrafluoroethylene (Reprinted with permission from John Wiley & Sons)

Table 1.2 Factors affecting the outcomes achieved with guided tissue regeneration in the treatment of intrabony lesions

Factors related to the case selection		Observation	References
General factors related to the patient	Patient compliance in self-performed plaque control	The ability to maintain high levels of plaque control has also been associated with improved outcomes	Tonetti et al. (1995, 1996), Cortellini et al. (1994, 1999b), Machtei et al. (1994), Ehmke et al. (2003)
	Level of residual periodontal infection in the remaining dentition	The level of residual periodontal infection in the dentition can be evaluated clinically as the percentage of sites with bleeding on probing, or microbiologically as the persistence of periodontal pathogens after completion of initial therapy	Nowzari et al. (1996), Tonetti et al. (1993), Machtei et al. (1994)
	Cigarette smoking	Cigarette smoking, has been associated with reduced outcomes	Trombelli and Scabbia (1997), Ehmke et al. (2003), Stavropoulos et al. (2004a), Mayfield et al. (1998), Loos et al. (2002), Machtei et al. (2003), Tonetti et al. (1995), Bowers et al. (2003), Trombelli et al. (1997)
	Systemic conditions	Diabetes, immunosuppression and stress could negatively interfere with the clinical outcomes	Mattson et al. (1998), Kornman and Robertson (2000)

Table 1.2 (continued)

Factors related to the case selection		Observation	References
Local factors related to the defect	The depth and width of the infrabony component of the defect	Narrow and deep infrabony defects respond radiographically and are to some extent clinically more favorable to GTR therapy than are wide and shallow defects	Tonetti et al. (1996, 1993), Laurell et al. (1998), Garrett et al. (1988), Cortellini et al. (1993c), Steffensen and Webert (1989), Cortellini et al. (1998), Ehmke et al. (2003), Eickholz et al. (2004a), Klein et al. (2001)
	The number of bony walls of the defect	Three-wall defects are achieving the best results	Handelsman et al. (1991), Becker and Becker (1993), Selvig et al. (1993)
	Gingival tissue thickness	Thin tissues are achieving significantly less clinical improvements and percentages of root coverage	Harris (1997), Anderegg et al. (1995)
Factors related to the procedural technique	Abilities to create and maintain the necessary space for regeneration,	Cell occlusion and space provision may significantly influence the magnitude of alveolar bone regeneration in conjunction with guided tissue regeneration	Polimeni et al. (2004a, b, 2005)
	Different surgical approaches to access the interdental spaces, to preserve tissues and to protect the area of regeneration, are associated with different outcomes.	Different ability in tissue management, membrane manipulation, attention to blood supply, suturing technique and other factors may play a major role in a difficult procedure such as guided tissue regeneration. Different surgical approaches were used: – The conventional flap approach (access flap or modified Widman flap) – The modified papilla preservation technique – The simplified papilla preservation flap – The papilla amplification flap	Becker et al. (1988), Cortellini et al. (1990, 1999b, 1999a, 1993c), Handelsman et al. (1991), Zucchelli and De Sanctis (2005)

debridement, bone replacement grafts, coronally repositioned flaps and guided tissue regeneration barriers (Sanz and Giovannoli 2000; Carnevale et al. 1997). The clinical responses of furcation defects to GTR depend on their extent and location. Evidence indicates that GTR can be successfully used only in the treatment of class II mandibular furcations and has a limited clinical effect on class II maxillary furcations (Villar and Cochran 2010).

1.3.1 Histological Studies

Histological evaluation remains the only reliable method of determining the nature of the attachment apparatus resulting from regenerative periodontal therapy and has been used in studies on animal models, mostly beagle dogs, nonhuman primates and sheeps (Keles et al. 2009; da Silva et al. 2008; de Andrade et al. 2007; Christgau et al. 2007; Gonçalves et al.

2006a, b; Regazzini et al. 2004; Donos et al. 2003; Cetiner et al. 2004; Rossa et al. 2000; Araújo et al. 1996, 1997, 1998, 1999; Sculean et al. 1998; Wikesjö et al. 1998; Danesh-Meyer et al. 1997; Lundgren et al. 1995; Park et al. 1995; Lindhe et al. 1995; Pontoriero et al. 1992; Caffesse et al. 1990).

1.3.2 Clinical Studies

The invasion of the furcation areas of multirooted teeth by periodontitis represents a serious complication in periodontal therapy. Regeneration of furcation defects has been reported following a variety of surgical approaches involving root surface conditioning, often combined with coronally advanced flap procedures, the placement of bone grafts or bone substitute implants or the use of organic or synthetic barrier membranes (guided tissue regeneration) (Karring and Cortellini 1999).

1.3.2.1 Therapeutic End Points of Success

One of the most important factors in determining the outcome of regenerative therapy is the ability to determine and analyze the types of healing outcomes that result. These outcomes can be relevant to the patient, to the defect treated or to the tissue healing response (Sanz and Giovannoli 2000).

Therefore, the main clinical endpoint of any given therapy to treat these lesions would be the full closure of the furcation. Unfortunately, very few studies reported the frequency of furcation closure and thus the predictability of the procedures. From a clinical point of view, the complete elimination of the inter-radicular defect appears to be an important outcome, establishing anatomic conditions that facilitate optimal plaque control (Figs. 1.5 and 1.6). Most of the studies have scheduled the surgical re-entry procedure 6 months postsurgery. It may be speculated that this may be too early for a final evaluation of bone fill in furcation defects (Jepsen et al. 2002).

If this objective could not be attained completely, then the secondary objective would be the conversion of a deep furcation lesion into a shallower one, thus converting a class II or III lesion into a class I lesion. Other possible surrogate endpoints will be gains in clinical attachment, mainly horizontal, together with gains in bone height. To assess these outcomes, most of the studies have used clinical or radiological methods. These methods have generally included

assessments of gingival inflammation expressed as bleeding on probing, periodontal probing for the evaluation of soft tissue changes and re-entry bone fill or radiographic bone changes for hard tissue evaluation (Sanz and Giovannoli 2000).

Relevant patient-related outcome measurements have seldom been used in regenerative therapy studies and therefore efficacy issues related to long-term stability of the patient's dentition, patient's comfort and patient's absence of pain or sensitivity are seldom measured. Therefore, the real impact of these regenerative techniques on the patient and the patient's perception of these types of therapies are unknown (Sanz and Giovannoli 2000).

1.3.2.2 Class II Mandibular Furcations

Although a certain variability occurs in the clinical outcomes, the use of barrier membranes generally demonstrates significant clinical advantages compared to debridement in mandibular degree II furcation defects. The reported average gains of vertical and horizontal clinical attachment in degree II defects treated with guided tissue regeneration ranged from 0.7 to 3.7 mm and from 0.8 to 3.7 mm, respectively. The corresponding parameters for the control defects ranged from -0.7 to 1.7 mm and from 0.1 to 2.1 mm. The variations in vertical and horizontal bone fill are 0.2–2.9 mm and 0.2–4.5 mm for furcations treated

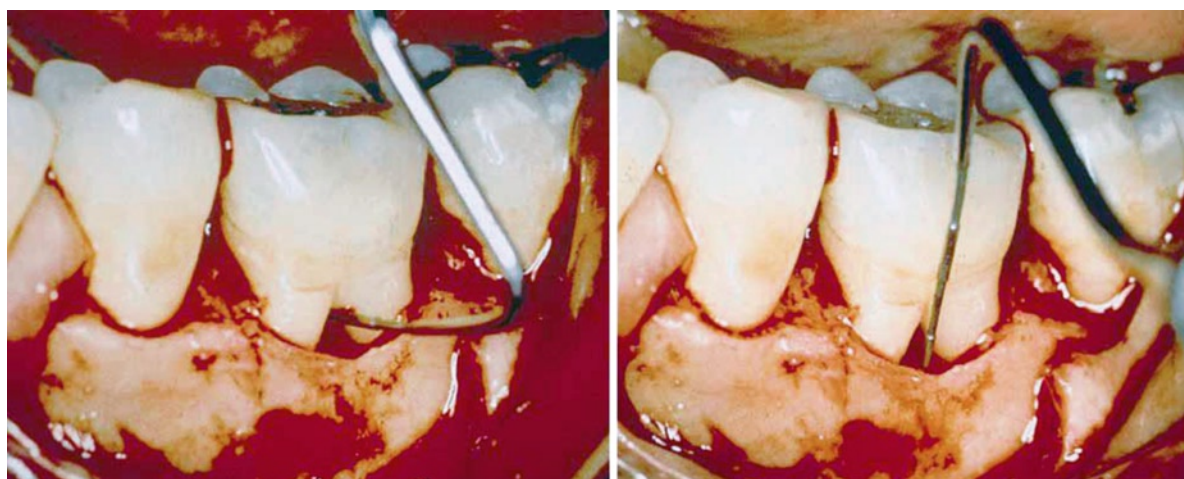


Fig. 1.5 Horizontal (*left*) and vertical (*right*) probing after flap elevation and debridement (Sanz and Giovannoli 2000. Reprinted with permission from John Wiley & Sons)

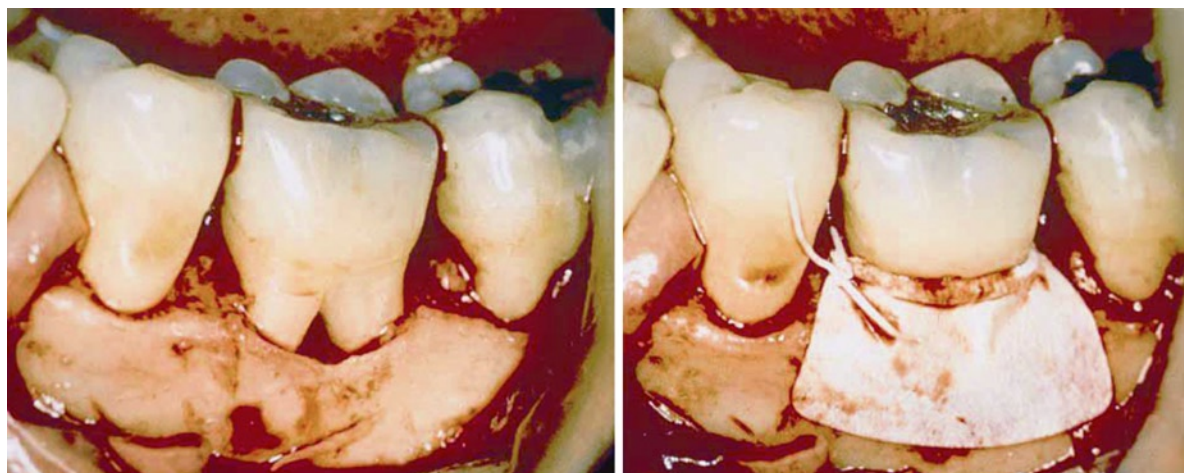


Fig. 1.6 A nonresorbable expanded polytetrafluoroethylene membrane positioned to cover the furcation entrance (Sanz and Giovannoli 2000. Reprinted with permission from John Wiley & Sons)

with guided tissue regeneration and -1.3 to 1.5 mm and -0.2 to 1.3 mm for the controls (Karring and Cortellini 1999).

The observed differences in favor of guided tissue regeneration when compared with debridement in the treatment of mandibular degree II furcation defects are supported by the results of a meta-analysis performed by. Guided tissue regeneration, used alone or in combination with bone replacement grafts, had the highest overall ranking. Mean reduction in probing depths and gains in vertical and horizontal attachment levels were all statistically significant at 6 months. Similar results were obtained in the 12-month studies. Compared to flap debridement, guided tissue regeneration resulted in greater reduction in probing depths and greater gains in vertical and horizontal attachment levels. Guided tissue regeneration provided almost identical results whether used with or without root conditioning, suggesting that root conditioning does not offer an adjunctive effect. A combination of guided tissue regeneration and bone replacement grafts yielded better results than did guided tissue regeneration alone in reducing probing depths and increasing vertical attachment levels.

The results of the meta-analysis performed by Jepsen et al. (2002) of four included studies (Lekovic et al. 1989, 1991; Wang et al. 1994; Mellonig et al. 1994) that had addressed the change in horizontal furcation depth (open assessment at re-entry) outcome showed a statistically significantly greater reduction in horizontal furcation depth for test groups compared

with open flap debridement. The weighted mean difference between test and control was 1.51 mm (95% CI: 0.39 , 2.62 ; $P < 0.001$). Jepsen et al. (2002) considered two studies on mandibular class II furcations employing change in horizontal probing attachment level as an outcome (Pontoriero et al. 1988; Wang et al. 1994). Because one study reported results for buccal and lingual furcations separately (Pontoriero et al. 1988), sensitivity analyses were undertaken. Both meta-analyses demonstrated a greater gain in horizontal probing attachment for GTR. When buccal data was included the weighted mean difference between test and control amounted to 1.31 mm (95% CI: -0.44 , 3.05 ; $P = 0.001$). The inclusion of the lingual data resulted in a weighted mean difference of 0.90 mm (95% CI: 0.50 , 1.30 ; $P = 0.131$) (Jepsen et al. 2002).

Six studies that showed changes in vertical probing attachment level as an outcome (Mombelli et al. 1996; Mellonig et al. 1994; Wang et al. 1994; Pontoriero et al. 1988; Lekovic et al. 1989, 1991) were analyzed by Jepsen et al. (2002). Because one study presented separate data for buccal and lingual sites, sensitivity analyses were undertaken. Both meta-analyses led to similar results indicating significantly greater gain in vertical probing attachment following GTR therapy. When the buccal data was included a weighted mean difference of 1.77 mm (95% CI: 0.63 , 2.91 ; $P < 0.001$) was evident. The inclusion of the lingual data revealed a weighted mean difference of 1.73 mm (95% CI: 0.61 , 2.85 ; $P < 0.001$) (Jepsen et al. 2002).

1.3.2.3 Class II Maxillary Furcations

Significant improvement beyond debridement was also observed in degree II buccal maxillary furcation defects following guided tissue regeneration but not to the same extent as in mandibular defects (Pontoriero and Lindhe 1995b; Karring and Cortellini 1999). From the summarized studies (Mellonig et al. 1994; Metzler et al. 1991; Pontoriero and Lindhe 1995b) by Sanz and Giovannoli (2000), it was concluded that the placement of a barrier membrane in this clinical situation does not add any benefit when compared with the standard treatment (open flap debridement). Both vertical and horizontal attachment gains are of a magnitude of within 1 mm, and in no case is there any furcation closed or any significant difference between the guided tissue regeneration and open flap debridement. Therefore, placement of a barrier membrane should not be indicated in the treatment of maxillary molars with furcation involvement.

In contrast, the meta-analysis performed by Jepsen et al. (2002) of four included studies (Metzler et al. 1991; Avera et al. 1998; Mellonig et al. 1994; Pontoriero and Lindhe 1995b) that presented data for maxillary furcations that had addressed the change in horizontal furcation depth (open assessment at re-entry) outcome revealed a limited but statistically significant greater reduction in horizontal furcation depth for test groups compared with open flap debridement. The weighted mean difference between test and control was 1.05 mm (95% CI: 0.46, 1.64; $P < 0.001$). Analysis of two studies that reported probing attachment level changes in mesial furcations (Pontoriero and Lindhe 1995b; Avera et al. 1998) resulted that GTR provided no additional gains in clinical attachment in class II maxillary furcations (weighted mean difference of 0.76 mm; 95% CI: 0.29, 1.22; $P = 0.188$). From the results of another two studies that had not specified the location of the furcations (Metzler et al. 1991; Mellonig et al. 1994), a weighted mean difference of 0.82 mm (95% CI: 0.47, 1.18; $P = 0.716$) favoring the test therapy could be calculated (Jepsen et al. 2002). Class II maxillary furcation lesions treated with GTR failed also to consistently show improved reduction in vertical probing depths when compared with sites treated with open flap debridement alone (mean difference, 1.42 mm; 95% CI: 0.28, 2.55; $P = 0.398$) (Jepsen et al. 2002).

1.3.2.4 Class III Furcations

Although some degree of closure could be attained occasionally in class III furcations, the majority of the treated defects are still through and through at re-entry after 1 year, this event being highly unpredictable (Karring and Cortellini 1999).

Jepsen et al. (2002) reported that in class III furcation defects, only 38% were found to be closed in mandibular teeth after GTR therapy, whereas no maxillary furcation defect and none of the mandibular and maxillary control defects with open flap debridement were closed (all closed assessments) (Pontoriero et al. 1989; Pontoriero and Lindhe 1995a).

1.3.2.5 Comparative Studies Between Treatment of Furcation Defects with Non-bioresorbable and Bioresorbable Materials

As reviewed by Sanz and Giovannoli (2000), guided tissue regeneration procedures in the treatment of furcation defects demonstrate similar outcomes when different membrane barrier materials were compared (Black et al. 1994; Blumenthal 1993; Bouchard et al. 1993, 1997; Caffesse et al. 1997; Christgau et al. 1995; Garrett et al. 1997; Hugoson et al. 1995; Yukna 1992). The mean gain in vertical and horizontal clinical attachment for mandibular class II furcations treated with non-bioresorbable barriers ranged from 0.0 to 1.3 mm and from 0.8 to 1.8 mm, respectively as compared with 0.0–1.8 mm and 1.5–2.5 mm for defects treated with bioresorbable barrier membranes. The ranges in vertical and horizontal bone fill are 0.4–1.0 mm and 1.0–2.2 mm for non-bioresorbable membranes versus 0.8–1.6 mm and 1.5–2.5 mm for defects treated with bioresorbable materials (Karring and Cortellini 1999).

Murphy and Gunsolley (2003) examined the outcome variables for vertical probing attachment level and vertical probing depth reduction; eight study arms from seven studies directly compared the use of ePTFE to a bioresorbable barrier in the treatment of furcation defects (Garrett et al. 1997; Hugoson et al. 1995; Eickholz et al. 1998; Bouchard et al. 1997; Blumenthal 1993; Dos Anjos et al. 1998; Black et al. 1994). When examining the combined polymeric barrier groupings, a statistical difference in favor of bioabsorbable barrier

types over ePTFE barriers could be detected for vertical probing attachment level outcome measures ($P=0.0004$ and $P=0.018$, respectively), but not for vertical probing depth reduction ($P=0.152$) (Murphy and Gunsolley 2003).

1.3.2.6 The Results of Comparative Studies Between Guided Tissue Regeneration Treatment of Furcation Defects with or without the Adjunctive Use of Other Regenerative Techniques

As reviewed by Sculean et al. (2008), the combination barrier membranes and grafting materials for the treatment of furcation defects was evaluated in three canine studies (Caffesse et al. 1993; Lekovic and Kenney 1993; Deliberador et al. 2006). No additional benefits of combination treatments were detected (Sculean et al. 2008).

Sanz and Giovannoli (2000) considered that the results obtained in controlled studies demonstrate that the use of bone replacement grafts together with barrier membranes is of limited significant additional benefit, if any, to the use of membranes alone. Although a significant clinical benefit has been reported in some studies, this is unpredictable and does not demonstrate an improved tissue healing response. Therefore, the use of replacement grafts to improve the results of guided tissue regenerative therapy is not clearly justified (Sanz and Giovannoli 2000).

Similarly, the results of comparative studies between guided tissue regeneration treatment of furcation defects with or without the adjunctive use of other regenerative techniques were summarized by Karring and Cortellini (1999). The average gains of vertical clinical attachment in defects treated with guided tissue regeneration alone ranged from -0.2 to 2.4 mm, compared to 0.8 – 4.3 mm for the combined treatment. The ranges in vertical and horizontal bone fill are 0.1 – 3.8 mm and 0.1 – 3.1 mm, respectively, for guided tissue regeneration alone versus 2.3 – 5.1 mm and 1.6 – 4.2 mm for the combined treatment. The results indicate that an added benefit may be obtained by the use of grafting materials in combination with barrier membranes for the treatment of mandibular degree II furcations (Karring and Cortellini 1999).

Using the outcome variable of vertical probing attachment level, Murphy and Gunsolley (2003) also

examined the effect of the addition of an augmentation material under the physical barrier. Collectively reviewing all barriers, vertical probing attachment level was significantly enhanced by the addition of a particulate bone graft ($P=0.039$). As a subgroup, ePTFE plus a particulate graft resulted in a significantly greater gain in vertical probing attachment level as compared to ePTFE alone ($P<0.05$). Polymeric or cellulose barrier treatments were not enhanced by the use of a graft. Vertical probing depth reduction was also enhanced by the addition of a particulate graft ($P=0.004$) when all barriers were collectively reviewed. Again, the ePTFE grouping demonstrated a significant advantage ($P<0.05$) (Murphy and Gunsolley 2003).

1.3.2.7 Efficacy of Root-Conditioning Agents in Conjunction with Guided Tissue Regeneration in the Treatment of Furcation Lesions

Several studies reported that guided tissue regeneration of furcation defects provided almost identical results whether used with or without root conditioning, suggesting that root conditioning does not offer an adjunctive effect (Parashis and Mitsis 1993; Machtei et al. 1993).

1.3.2.8 Long-Term Evaluation

Using the principles of GTR, different authors reported survival rates ranging from 83.3% to 100% in the treatment of furcation-involved molars (observation period: 5–12 years) (Huynh-Ba et al. 2009).

Eickholz et al. (2001) treated nine pairs of contralateral class II furcation defects in nine patients with advanced periodontitis by applying the principles of GTR. Horizontal attachment gain achieved after GTR therapy in class II furcations was stable after 5 years in 16 of 18 defects (Eickholz et al. 2001) and after 10 years in 15 of 18 defects (83%) (Eickholz et al. 2006).

Five hundred and five molars in 71 patients (mean age 46 years; 40 females) were evaluated by Dannewitz et al. (2006) after at least 5 years of supportive periodontal therapy. Only one tooth out of 57 furcation-involved molars treated according to the principles of GTR was lost, yielding a survival rate of 98.1% (Dannewitz et al. 2006).

1.3.2.9 Factors Affecting the Outcomes Achieved with Guided Tissue Regeneration in the Treatment of Furcation Lesions

It has been shown that the use of the guided tissue regeneration therapeutic approach in the treatment of furcation defects results in a great variability of clinical outcomes. The variability in these clinical results has been explained in the literature through a variety of factors which may be related either to the case selection or to the therapeutic procedure or both (Tonetti et al. 1993; Sanz and Giovanolli 2000).

Several types of factors can be considered in the diagnosis and selection of a case for guided tissue regeneration therapy: general factors related to the patient and local factors related to the defect (Sanz and Giovanolli 2000; Novaes et al. 2005) (Table 1.3).

1.4 Summary of Studies Evaluating the Efficacy of GTR in the Treatment of Gingival Recession

Gingival recession and root exposure represent a therapeutic problem to the clinician. Patients often complain about poor esthetics and root sensitivity, and root caries and erosion are oftentimes associated with long-lasting recessions. Ideally, surgical treatment of gingival recession defects should fully restore the anatomy of the mucogingival complex. This implies regeneration of the attachment apparatus of the tooth, including cementum with inserting connective tissue fibers, and alveolar bone, as well as recreation of topographic relationships between the keratinized tissue and the alveolar mucosa that are functionally and esthetically acceptable to the patient (Trombelli 1999).

1.4.1 Histological Studies

Several experimental studies in dogs (Casati et al. 2000; Cortellini et al. 1991; Casati et al. 2000; Lee et al. 2002; Sallum et al. 2004; Papageorgiou et al. 2009) and non-human primates (Gottlow et al. 1990; Graziani et al. 2005) showed that periodontal regeneration and root

coverage may be obtained after a GTR procedure. In monkeys, the amount of new attachment formation was on the average 74.3% of the defect height in the test teeth, which corresponded to 100% of the membrane-covered root portion. Newly formed connective tissue attachment in the controls amounted to an average of 36.9% of the defect height (Gottlow et al. 1990).

Evidence of periodontal regeneration following root coverage procedures has also been provided from human biopsies (Trombelli 1999). Cortellini et al. (1993a) demonstrated, histologically, that the root coverage of a recession (8 mm deep with a pocket depth of 1 mm and no keratinized tissue) obtained with an ePTFE membrane included new connective tissue attachment (3.66 mm) as well as newly formed cementum (2.48 mm) and bone growth (1.84 mm). The crestal bone level after treatment was located coronal to the preoperative location of the gingival margin.

Different space-making solutions also have been used in combination with nonresorbable membranes (e.g., titanium-reinforced, gold bar-reinforced, and gold frame-reinforced membranes) to increase the percentage of root coverage with GTR (Kassab et al. 2010). Parma-Benfenati and Tinti (1998) demonstrated that the coronal extent of the new attachment and new facial bone, 9 months after guided tissue regeneration treatment, were located coronal to the preoperative location, in a root surface previously exposed by a deep, long-standing recession. Similar results were reported by Tinti et al. (1993).

1.4.2 Clinical Studies

The long-term effects of GTR in the treatment of gingival recessions were evaluated in a number of case reports and controlled clinical trials, summarized by several reviews and meta-analysis (Table 1.4).

1.4.2.1 GTR-Based Root Coverage

Al-Hamdan et al. (2003) revealed that GTR root coverage resulted in significant gain of CAL equal to 3.1 ± 1.2 mm ($P < 0.05$). In addition, keratinized gingiva was significantly increased by 1.0 ± 0.9 mm. On average, GTR root coverage resulted in $75.0 \pm 11.0\%$ root coverage and complete root coverage in $42.0 \pm 19.8\%$ of cases.

Table 1.3 Factors affecting the outcomes achieved with guided tissue regeneration in the treatment of furcation lesions

Factors related to the case selection		Observation	References
General factors related to the patient	Patient compliance in self-performed plaque control	Significant better clinical attachment level gains was demonstrated in patients with optimal levels of plaque control compared with patients with poor oral hygiene.	Cortellini et al. (1994), Sanz and Giovannoli (2000)
	Level of residual periodontal infection in the remaining dentition	In patients with high levels of residual infection, the obtained attachment gains are significantly lower.	Sanz and Giovannoli (2000), Machtei et al. (1994), Tonetti et al. (1993)
	Cigarette smoking	The success rate was significantly lower in smokers and the outcome for 80% of these cases was considered as failure	Rosenberg and cutler (1994), Tonetti et al. (1995), , Sanz and Giovannoli (2000), Machtei et al. (2003), Bowers et al. (2003)
	Systemic diseases	The proportion of class II residual defects was significantly higher among smokers than non-smokers (62.5% vs. 14.3%, respectively). A negative prognosis might be anticipated in patients with insulin-dependent diabetes mellitus, HIV-positive patients with other clinical or immunological deficiencies, in patients with rheumatoid arthritis, and other immune-complex diseases. High doses of irradiation in patients with a history of head and neck tumors may be also detrimental.	Novaes et al. (2005)
Local factors	Furcal anatomy	The furcal anatomy-related factors are the presence of cervical enamel projection, enamel pearls, root or root trunk concavities, bifurcation ridge, accessory canals, furcation entrance dimension and length of root trunk. The presence of a long root trunk facilitates the placement of the barrier membrane under the cementoamel junction, thus achieving full coverage of the furcation defect and the placement of the replacement flap fully covering the membrane.	Novaes et al. (2005), Sanz and Giovannoli (2000), Bower (1979)
	Defect morphology	The predictability of the regenerative procedure improves if the defect present a deep vertical component in the defect while maintaining the level of interproximal bone level close to the cementoamel junction. This facilitates the retention of the membrane in a proper position and allows for the coronal replacement of the flap and full coverage of the membrane. Furcations with vertical or horizontal bone loss of 5 mm or greater responded with the lowest frequency of complete clinical closure.	Sanz and Giovannoli (2000), Jepsen et al. (2002), Bowers et al. (2003), Horwitz et al. (2004), Novaes et al. (2005)
	Thickness of gingival tissue	The amount and quality of the gingival tissue that will cover the membrane is also important. Inadequate gingival width and thin keratinized tissue should be analyzed because it can lead to gingival recession.	Novaes et al. (2005)

(continued)

Table 1.3 (continued)

Factors related to the case selection		Observation	References
Factors related to the procedural technique	Tooth mobility	Conflicting results exist concerning the effect that presurgical hypermobility has on surgical healing and, thus, on the post-therapeutic clinical outcome. In clinical practice, the question of whether to splint mobile teeth prior to regenerative therapy to improve the healing outcome often arises.	Cortellini et al. (2001), Trejo and Weltman (2004), Novaes et al. (2005)
	Careful flap design	Poor operative technique in membrane placement or surgical soft tissue management and failure to adequately cover the membrane can cause gingival recession and consequently membrane exposure.	Sanz and Giovannoli (2000), Novaes et al. (2005)
	Root surface preparation	All subgingival soft and hard deposits at the furcation area should be removed through mechanical root instrumentation. Since the width of the furcation entrance and the internal morphology of the intraradicular area may limit the access of the curettes for proper debridement, it must frequently be complemented with ultrasonic and rotary instruments.	Sanz and Giovannoli (2000)
	Selection and placement of the membrane barrier	Once the barrier material has been selected, this membrane must be trimmed and tailored to the defect morphology in order to adapt it closely to the tooth and completely cover the entrance of the furcation area, extending approximately 3 mm of alveolar bone apical to the furcation. Ideally, its coronal border should be placed 2 mm below the cementoenamel junction without exposing the entrance of the furcation.	Sanz and Giovannoli (2000)
	Wound closure	Special attention should be given to the tensile strength of the sutures of the flap in order to avoid any collapse of the membrane into the defect, and also to cover the entire membrane surface.	Sanz and Giovannoli (2000), Novaes et al. (2005)
Postoperative factors	Postoperative care	Postoperative factors such as plaque control, membrane exposure, membrane retrieval and a regular supportive periodontal care program may also influence the final results. The patient should be instructed not to brush or to brush the operated area gently with an ultra-soft toothbrush and to rinse with chlorhexidine (0.2%) for a period of 4–6 weeks. If a nonresorbable barrier has been used, it should be removed after 4–6 weeks.	Sanz and Giovannoli (2000), Novaes et al. (2005)
	Second-stage surgery	This minor surgical procedure is only performed if a nonresorbable barrier has been used. To gain access to the barrier material, a small incision is made, extending one tooth mesial and distal of the border of the barrier. The soft tissue flap is gently reflected and the barrier material dissected free from the flap using a sharp blade. During this procedure, it is essential not to compromise the newly regenerated tissue and bone gain.	Tonetti et al. (1993), Sanz and Giovannoli (2000), Novaes et al. (2005)

Table 1.4 Systematic reviews and meta-analysis on the efficacy GTR in the treatment of gingival recessions

Authors	Studies included	Conclusions
Danesh-Meyer and Wikesjö (2001)	Reports published in the English language from 1985 to 2000 evaluating GTR in gingival recession defects in human subjects	It has been shown that GTR may be used for reconstruction of gingival recession defects. Importantly it has not been shown that GTR provides an added clinical benefit for the patient treatment planned for reconstruction of gingival recession defects. i.e., GTR does not appear to offer a significant advantage over mucogingival procedures such as the connective tissue graft or the advanced flap procedure. It is imperative to recognize inherent technical difficulties associated with GTR including primary wound closure and secondary membrane exposure: membrane exposures being negatively correlated to desired clinical outcomes. Also, membrane exposures appear consistently more common in smokers than in non-smokers.
Roccuzzo et al. (2002)	20 randomized and controlled trials, as well as case series of at least 6 months' follow-up, up to and including April 2001	Regarding recession reduction, a limited but statistically significant greater benefit was found for connective tissue graft compared with GTR (weighted mean difference: 0.43 mm, 95% CI: 0.62–0.23). No differences were found comparing either GTR with coronally advanced flap or resorbable versus non-resorbable GTR barriers.
Al-Hamdan et al. (2003)	40 studies were identified that used GTR approaches to treat gingival recession from January 1990 to October 2001. Studies length: at least 6 months	Guided tissue regeneration-based root coverage resulted in an average of 74% recession depth reduction, 41% complete root coverage, 3 mm attachment level gain, and 1 mm keratinized gingiva gain. Both guided tissue regeneration-based root coverage and conventional mucogingival surgery produced significant ($P < 0.05$) improvement compared to baseline measurements. Compared to guided tissue regeneration-based root coverage, conventional mucogingival surgery resulted in significantly ($P < 0.05$) increased keratinized gingiva (2.1 mm vs. 1.1 mm), root coverage (81% vs. 74%), and percentage of defects with complete root coverage (55% vs. 41%). Use of absorbable membranes, root conditioning, shallow pretreatment recession (< 4 mm), and corporate sponsorship all resulted in significantly ($P < 0.05$) improved percentages of sites with complete root coverage but had no effect on other parameters.
Oates et al. (2003)	32 articles published by April 2002 (total study population: 687) met the criteria for RCTs: 11 (population: 286) related to various autogenous soft tissue augmentation procedures; 18 (population: 360) to GTR; and 3 (population: 41) to allogenic soft tissue augmentation.	Soft tissue augmentation procedures are effective means of obtaining root coverage. Meta-analysis identified greater gains in both root coverage and keratinized tissue width for connective tissue graft procedures compared to GTR.
Cairo et al. (2008)	Randomized clinical trials on treatment of Miller class I and II gingival recessions with at least 6 months of follow-up	A total of 794 Miller class I and II gingival recessions in 530 patients from 25 RCTs were evaluated in this systematic review. CAF was associated with mean recession reduction and CRC. The addition of connective tissue graft (CTG) or EMD enhanced the clinical outcomes of CAF in terms of CRC, while BM did not. The results with respect to the adjunctive use of acellular dermal matrix were controversial.

(continued)

Table 1.4 (continued)

Authors	Studies included	Conclusions
Chambrone et al. (2010)	24 RCTs up to October 2008 with a duration ≥ 6 months that evaluated recession areas (Miller class I or II ≥ 3 mm) that were treated by means of periodontal plastic surgery procedures were included.	(1) Subepithelial connective tissue grafts, a coronally advanced flaps alone or associated with grafts or biomaterials (e.g., acellular dermal matrix grafts, enamel matrix protein, and subepithelial connective tissue grafts), and GTR may be used as root-coverage procedures for the treatment of recession-type defects. In cases where both root coverage and gain in the width of keratinized tissue are expected, the use of subepithelial connective tissue grafts seems to be more adequate. (2) Acellular dermal matrix grafts may be an alternative treatment in cases where subepithelial connective tissue grafts harvested from the palate are not sufficient to cover a recession area. (3) Root modification agents may be used for root conditioning; however, it is not evident that these products improve root coverage.

Oates et al. (2003) identified 18 studies in which GTR procedures were assessed for treatment of recession defects. The GTR procedures in these studies used either bioresorbable or nonresorbable materials. Evaluation of mean root coverage in 17 of these 18 studies utilizing GTR procedures found $76.4 \pm 11.3\%$ root coverage, with 100% root coverage at $33.1 \pm 20.4\%$ of the sites. Using GTR procedures, mean gains in CAL were 3.2 ± 1.14 mm based on 16 of the 18 studies. Changes in probing depths were minimal for all 18 studies (mean 0.53 ± 0.41 mm) and may be reflective of the shallow probing depth identified at baseline (mean: 1.54 mm) (Oates et al. 2003).

Cairo et al. (2008) revealed that the comparison of coronally advanced flap + barrier membrane versus coronally advanced flap showed no significant difference regarding complete root coverage (Lins et al. 2003) ($P=0.41$; OR = 0.58; 95% CI from 0.16 to 2.08), recession reduction (studies included: Amarante et al. 2000; Lins et al. 2003; Leknes et al. 2005) ($P=0.11$; mean difference = -0.27 mm; 95% CI from -0.60 to 0.06), CAL gain (studies included: Amarante et al. 2000; Lins et al. 2003; Leknes et al. 2005), although a trend favoring CAF was detected ($P=0.06$; mean difference = -0.33 mm; 95% CI from -0.68 to 0.02). Regarding keratinized tissue gain, meta-analysis reported no significant difference between the two types of surgical procedures ($P=0.30$; mean difference = 0.15 mm; 95% CI from -0.13 to 0.42).

In the systematic review performed by Chambrone et al. (2010), the percentage of complete root coverage varied from 33.3% (Dodge et al. 2000) to 53.3% (Paolantonio et al. 2002) for GTR bioabsorbable

membranes, and 28.0% (Zucchelli et al. 1998) to 41.6% (Roccuzzo et al. 1996) for GTR non-resorbable membranes. Although no statistical differences were found among procedures, a substantial number of patients for the GTR bioabsorbable membrane and GTR non-resorbable membrane procedures, 12 of 30 (40%) and 10 of 30 (33.33%) patients, respectively, did not achieve complete root coverage. Regarding mean root coverage, this outcome varied from 62.5% (Matarasso et al. 1998) to 73.7% (Dodge et al. 2000) for GTR bioabsorbable membranes to 82.4% (Roccuzzo et al. 1996) for GTR non-resorbable membrane procedures.

1.4.2.2 GTR-Based Root Coverage Versus Conventional Mucogingival Surgery

The choice of GTR or gingival grafting to obtain root coverage has been a controversial subject (Kassab et al. 2010).

Studies comparing GTR and subepithelial connective tissue graft, as reviewed by Danesh-Meyer and Wikesjö (2001), suggest that both protocols offer means of obtaining root coverage of gingival recession defects (Figs. 1.1 and 1.2). It appears, however, that the subepithelial connective tissue graft protocol provides improved root coverage over that observed following GTR. The subepithelial connective tissue graft protocol also results in a substantially increased keratinized gingiva compared to only incremental improvements following GTR. A possible explanation for these observations may be the occurrence of membrane exposures and ensuing compromised wound healing following GTR (Danesh-Meyer and Wikesjö 2001).

Roccuzzo et al. (2002) demonstrated that placement of a connective tissue graft was significantly better than GTR in reducing recession, weighted mean difference 0.43 mm (95% CI: 0.62, 0.23, chi-square for heterogeneity 7.8 (df = 5) $P=0.17$). Regarding attachment gain, the analysis for GTR versus connective tissue graft only just missed statistical significance (WMD 0.21 mm, 95% CI: -0.055, 0.43 chi-square for heterogeneity = 2.85 (df = 4) $P=0.58$). The largest percentages of cases with complete root coverage for each treatment were GTR with non-resorbable membranes 46.7% sites (Jepsen et al. 1998), GTR with resorbable membranes 41.6% sites (Roccuzzo et al. 1996), connective tissue graft 83.3% sites (Tatakis and Trombelli 2000), free gingival graft 44.4% sites (Borghetti and Gardella 1990) and coronally advanced flap (with enamel matrix derivative) 64% sites (Modica et al. 2000).

In his review, Al-Hamdan et al. (2003) summarized results of 18 studies that compared GTR with conventional mucogingival surgery. The conventional mucogingival surgery group comprised 271 sites and the GTRC group comprised 272 sites. Both procedures produced statistically significant ($P<0.05$) decreases in recession depth. Conventional mucogingival surgery reduced recession depth from a pretreatment average of 4.0 ± 0.9 mm to 0.8 ± 0.7 mm posttreatment, corresponding to $81.0\pm6.7\%$ root coverage. With GTRC, average pretreatment recession of 4.2 ± 1.0 mm was reduced to 1.2 ± 0.9 mm, corresponding to $72.0\pm9.1\%$ root coverage. Conventional mucogingival surgery yielded complete root coverage in $55.3\pm17.8\%$ of treated cases, while GTRC resulted in complete root coverage in only $41.3\pm19.4\%$ of the treated cases. These differences were statistically significant ($P<0.05$). Both conventional mucogingival surgery and GTRC resulted in significant gains of clinical attachment (2.7 ± 1.2 and 3.1 ± 1.3 mm, $P<0.05$), but there was no difference between the two groups. Conventional mucogingival surgery increased width of keratinized gingival by 2.1 ± 1.1 mm, while GTRC increased keratinized gingiva width by only 1.1 ± 0.8 mm. This difference was statistically significant (Al-Hamdan et al. 2003).

Meta-analysis performed by Oates et al. (2003) identified greater gains in both root coverage (2.90 ± 1.10 mm vs. 2.56 ± 1.09 mm) and keratinized tissue width (1.33 ± 1.19 vs. 0.48 ± 1.03 mm) for connective tissue graft procedures compared to GTR.

Cairo et al. (2008) reported no significant difference in comparing coronally advanced flap + barrier membrane versus coronally advanced flap + connective tissue graft (6 RCTs included: Borghetti et al. 1999; Jepsen et al. 1998; Romagna-Genon 2001; Tatakis and Trombelli 2000; Trombelli et al. 1998; Wang et al. 2001; Zucchelli et al. 1998) regarding complete root coverage, although a trend favoring coronally advanced flap + connective tissue graft was detected ($P=0.06$; OR=0.45; 95% CI from 0.20 to 1.04) by Cairo et al. (2008). Meta-analyses reported better results for coronally advanced flap + connective tissue graft for recession reduction ($P=0.008$; mean difference = -0.38 mm; 95% CI from -0.65 to -0.10), but no significant difference for CAL gain ($P=0.73$; mean difference = -0.05 mm; 95% CI from -0.32 to 0.22). Regarding keratinized tissue gain, comparison resulted in better outcomes for coronally advanced flap + connective tissue graft ($P=0.004$; mean difference = -1.18 mm; 95% CI from -1.98 to -0.39). For this comparison, the test for heterogeneity was found to be statistically significant ($P<0.00001$).

In the recent meta-analysis performed by Chambrone et al. (2010), with respect to gingival recession and keratinized tissue changes, there was a statistically significantly greater reduction in gingival recession and greater gain in the width of keratinized tissue for subepithelial connective tissue grafts compared to GTR bioabsorbable membrane sites ($P<0.0001$). For keratinized tissue changes, there was also a significantly greater gain in the width of keratinized tissue for subepithelial connective tissue grafts compared to GTR bioabsorbable membranes associated with bone substitutes ($P<0.0001$). In addition, risk-ratio analyses were available for comparison, GTR bms versus SCTG (outcome 4.4). Although no statistical differences were found among procedures, for the SCTG and GTR bioabsorbable membrane procedures, 20 of 49 (40.81%) and 28 of 49 (57.14%) patients, respectively, did not achieve complete root coverage (Chambrone et al. 2010).

The long-term stability of root coverage (i.e., the reduction of recession depth) and esthetic results perceived by patients were significantly better 10 years after connective tissue graft surgery, statistically, than after GTR surgery using bioabsorbable barriers (Nickles et al. 2010). Six and 120 ± 12 months after receiving a connective tissue graft, statistically significant ($P<0.05$) root coverage was observed compared

to baseline root coverage (6 months: 3.07 ± 1.74 mm; 120 ± 12 months: 2.07 ± 1.89 mm). The GTR therapy resulted in statistically significant root coverage compared to baseline root coverage only after 6 months (2.28 ± 1.77 mm; $P < 0.05$). Both groups experienced a statistically significant loss of coverage from 6 to 120 ± 12 months (connective tissue graft: -1.0 ± 0.78 mm; GTR: -2.03 ± 2.24 mm). At 120 ± 12 months after connective tissue graft surgery, the stability of root coverage was statistically significantly better than 120 ± 12 months after GTR surgery ($P = 0.002$). The connective tissue graft caused more postsurgical discomfort ($P < 0.05$), but it resulted in a better treatment outcome ($P < 0.05$) than GTR as perceived by patients (Nickles et al. 2010).

1.4.2.3 Non-absorbable Membranes Versus Absorbable Membranes

As Danesh-Meyer and Wikesjö (2001) revealed, root coverage among the studies using nonresorbable membranes averaged 3.5 ± 0.7 mm. Attachment level gain averaged 4.0 ± 0.9 mm. Importantly, probing depths in the augmented sites remained shallow following the GTR protocol (Figs. 1.1 and 1.2). A limited mean increase in keratinized gingiva (0.6 ± 0.8 mm; Fig. 1.3) was observed among studies using nonresorbable membranes. Keratinized gingivas ranged from 1.0 to 1.9 mm pretreatment compared to 0.5–6.2 mm post-treatment (Fig. 1.9).

A disadvantage of nonresorbable GTR devices is the need for a second surgical procedure to remove the membrane, an obvious inconvenience to the patient and the clinician. Importantly, this second surgical procedure may disrupt any regenerated tissues including the connective tissue attachment to the root surface. These concerns have spurred the development and manufacture of bioresorbable GTR devices using tissue derivatives and organic polymer technologies. Root coverage among the studies using bioresorbable membranes averaged 2.8 ± 1.2 mm. Attachment level gain averaged 2.5 ± 1.3 mm. As observed for nonresorbable membranes, probing depths remained shallow following the GTR protocol (Figs. 1.7 and 1.8). Bioresorbable membranes appear less effective than the nonresorbable membrane technology in more limited gingival recession defects; however, this relative deficiency appears compensated in advanced defects. As observed for the nonresorbable membrane technology,

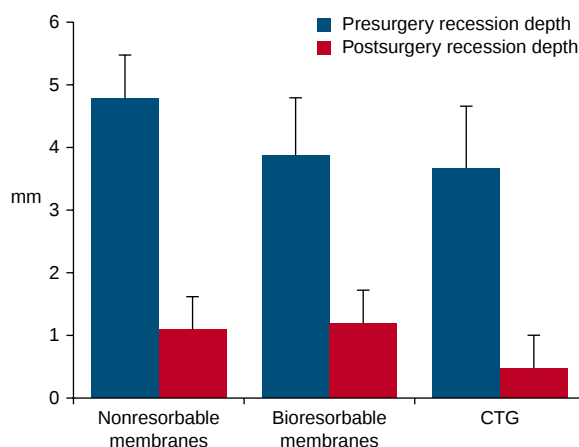


Fig. 1.7 Comparisons of pre- and posttreatment gingival recession depths for studies evaluating guided tissue regeneration (GTR) using nonresorbable or bioresorbable membranes, or subepithelial connective tissue graft (CTG) (Danesh-Meyer and Wikesjö 2001. Reprinted with permission from John Wiley & Sons)

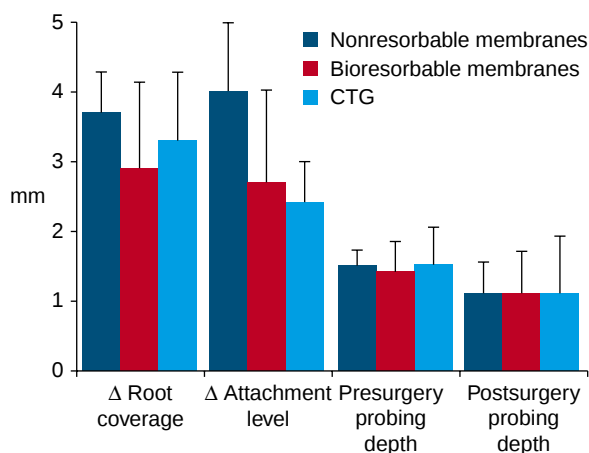


Fig. 1.8 Change in gingival root coverage and clinical attachment level, and pre- and posttreatment probing depths for studies evaluating guided tissue regeneration (GTR) using nonresorbable or bioresorbable membranes, or subepithelial connective tissue graft (CTG) (Danesh-Meyer and Wikesjö 2001. Reprinted with permission from John Wiley & Sons)

keratinized gingiva increases slightly following GTR using bioresorbable membranes. This increase, however, appears to be smaller than for nonresorbable membranes (Fig. 1.9), several studies actually reporting no effect or decreased keratinized gingiva post-treatment (Danesh-Meyer and Wikesjö 2001).

No significant differences were found by Roccuzzo et al. (2002) for gingival recession reduction between

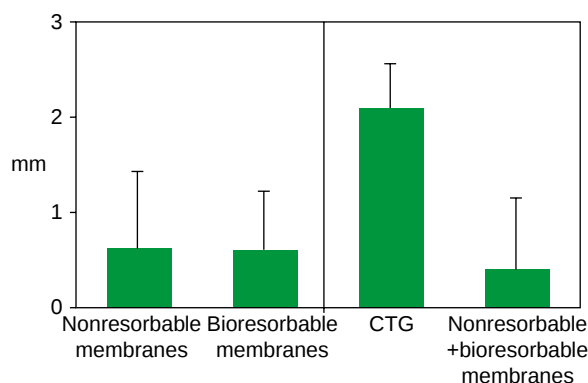


Fig. 1.9 Change in the width of the keratinized gingiva following guided tissue regeneration (GTR) and subepithelial connective tissue graft (CTG) (Danesh-Meyer and Wikesjö 2001. Reprinted with permission from John Wiley & Sons)

resorbable and non-resorbable membranes for GTR, weighted mean difference 0.27 (95% CI: -0.07, 0.60, chi-square for heterogeneity 1.57 (df = 1), $P=0.21$). No significant differences were observed in comparative studies between resorbable versus nonresorbable barriers regarding attachment level gain. Single study arms from RCTs and CCTs revealed that attachment level change for resorbable barriers was 2.84 mm (95% CI: 1.76, 3.93, chi-square for heterogeneity 261.6 (df = 6) $P=0.001$) and for nonresorbable membranes, 4.01 mm (95% CI: 2.96, 5.06, chi-square for heterogeneity 66.4 (df = 4) $P=0.001$) (Roccuzzo et al. 2002).

Al-Hamdan et al. (2003) considered that the type of the membrane used (nonabsorbable vs. absorbable) did not affect the PD reduction (0.4 ± 0.5 mm vs. 0.1 ± 0.4 mm), gain in CAL (3.2 ± 1.6 mm vs. 3.1 ± 1.0 mm), keratinized gingiva gain (1.2 ± 1.0 mm vs. 0.9 ± 0.9 mm) or percentage of root coverage ($74.3 \pm 5.7\%$ vs. $76.3 \pm 13.2\%$). Cases treated with absorbable membranes had a higher percentage of complete root coverage compared to nonabsorbable membranes ($45.0 \pm 20.9\%$ vs. $35.0 \pm 17.2\%$).

Similar results were reported by Chambrone et al. (2010). With respect to gingival recession (effect size 0.32; 95% CI: -0.03 to 0.68), clinical attachment level (effect size 0.15; 95% CI: -0.38 to 0.68) and keratinized tissue (effect size: 0.11; 95% CI: -0.29 to 0.51) changes, all comparisons failed to demonstrate significant differences among GTR procedures using nonabsorbable versus absorbable membranes (studies included: Roccuzzo et al. 1996; Zucchelli et al. 1998). The percentage of complete root coverage varied from 33.3% (Dodge et al. 2000) to 53.3% (Paolantonio et al.

2002) for GTR bioabsorbable membranes, and 28.0% (Zucchelli et al. 1998) to 41.6% (Roccuzzo et al. 1996) for GTR non-resorbable membranes. In addition, risk-ratio analyses for were available for GTR bms versus GTR nrms, outcome 5.4. Although no statistical differences were found among procedures, a substantial number of patients for the GTR bioabsorbable membrane and GTR non-resorbable membrane procedures, 12 of 30 (40%) and 10 of 30 (33.33%) patients, respectively, did not achieve complete root coverage. Regarding mean root coverage, this outcome varied from 62.5% (Matarasso et al. 1998) to 73.7% (Dodge et al. 2000) for GTR bioabsorbable membranes to 82.4% (Roccuzzo et al. 1996) for GTR non-resorbable membrane procedures (Chambrone et al. 2010).

1.4.2.4 Effect of Root Conditioning on GTR Root Coverage

Root surface conditioning was used in 16 studies with 281 recession defects compared to 31 studies with 412 recession defects that did not. There was no significant difference between the two groups with regard to all clinical parameters measured except percentage of sites with complete root coverage. The root surface conditioned group had a higher percentage of complete root coverage than sites treated without root conditioning agents ($51.7 \pm 22.4\%$ vs. $32.1 \pm 15.1\%$, respectively) (Al-Hamdan et al. 2003).

1.4.2.5 Effect of Bone Replacement Graft on GTR Root Coverage

Al-Hamdan et al. (2003) reported that the addition of bone replacement graft did not improve the percentage of root coverage ($79.6 \pm 16\%$ vs. $78.5 \pm 14.1\%$ with no BRG). Use of bone replacement graft has also no effect on posttreatment increase in width of keratinized gingiva (Al-Hamdan et al. 2003).

Similar results were reported by Chambrone et al. (2010). With respect to gingival recession (effect size: 0.46; 95% CI: -0.02 to 0.94), clinical attachment level (effect size: 0.72; 95% CI: -0.06 to 1.50) and keratinized tissue (effect size: 0.13; 95% CI: -0.12 to 0.37) changes, all comparisons failed to demonstrate significant differences among GTR procedures using absorbable membranes associated or not with bone substitutes (studies included: Dodge et al. 2000;

Paolantonio 2002). In addition, risk-ratio analyses were available for comparison of GTR bms with bone substitutes versus GTR bms, outcome 7.4. Although no statistical differences were found among procedures, a substantial number of patients for the GTR bioabsorbable membrane bone substitutes and GTR bioabsorbable membrane procedures, 14 of 27 (51.85%) and 10 of 27 (37.03%) patients, respectively, did not achieve complete root coverage. The mean root coverage outcome varied from 62.5% (Matarasso et al. 1998) to 73.7% (Dodge et al. 2000) for GTR bms, and 84.2% (Rosetti et al. 2000) to 89.9% (Dodge et al. 2000) for GTR bms associated with bone substitutes (Chambrone et al. 2010).

1.4.2.6 Effect of Pretreatment Recession Depth on GTR Root Coverage

Single study arms from RCTs and CCTs evaluated by Roccuzzo et al. (2002) showed that the change in recession following the placement of resorbable membranes was 2.85 mm (95% CI: 1.72, 3.99, chi-square for heterogeneity 439.3 (df = 7), $P < 0.001$) and, for nonresorbable membranes, we found a weighted mean difference of 3.70 mm (95% CI: 2.99, 4.41, chi-square for heterogeneity 36.4 (df = 4), $P < 0.001$). To explore the substantial heterogeneity, a meta-analysis regression on initial recession depth was conducted. This analysis explained some of the heterogeneity for GTR and suggested an increase of 0.99 mm (95% CI: 0.69, 1.31) and 0.67 mm (95% CI: 0.43, 0.90) improvement in recession for each additional 1 mm of recession depth, respectively, for resorbable and nonresorbable barriers ($P < 0.001$) (Roccuzzo et al. 2002).

The analyses performed by Al-Hamdan et al. (2003) on 21 studies with 363 recession defects with a mean pretreatment recession depth of <4 mm, and on 26 studies with 330 recession defects with a mean pretreatment recession depth of ≥ 4 mm revealed no difference between the two groups in terms of posttreatment recession depth reduction, percentage of root coverage, probing depth changes and gain of keratinized gingiva. Gain for CAL was significantly greater for deep recession defects compared to shallow recession defects (3.9 ± 1.4 mm vs. 2.2 ± 0.9 mm, $P < 0.05$). In contrast, shallow recession defects exhibited higher percentage of complete root coverage than deep defects ($51.2 \pm 22.0\%$ vs. $32.0 \pm 13.0\%$, respectively, $P < 0.05$).

1.4.2.7 Influence of Smoking on GTR Root Coverage

It was shown that smoking can affect the results obtained by periodontal plastic surgery procedures. Subepithelial connective tissue grafts resulted in 27.0–80.0% complete root coverage for nonsmokers and 0–25.0% for smokers. Similarly, coronally advanced flaps resulted in 20.0–55.1% complete root coverage for nonsmokers and 0–54.5% for smokers. For guided tissue regeneration, complete root coverage was 38.5% for nonsmokers and 11.1% for smokers. Additionally, nonsmokers exhibited significantly more sites with complete root coverage than did smokers ($P = 0.001$). (Chambrone et al. 2009; Trombelli and Scabbia 1997; Zucchelli et al. 1998) (Fig. 1.10).

1.4.2.8 Long-Term Effect of GTR in Gingival Recession Defects

Few studies reported a follow-up period >12 months (Chambrone et al. 2010; Rosetti et al. 2000; Trombelli et al. 2005; Nickles et al. 2010). The studies by Pini Prato et al. (1996) and by Scabbia and Trombelli (1998) suggest that root coverage following GTR in gingival recession defects remains stable over several years. Moreover, the studies suggest that once healing has successfully taken place, the patient's smoking status does not pose a risk for further gingival recession or attachment level loss in a compliant patient population. These observations are important considerations in the clinical recommendation of root coverage procedures (Danesh-Meyer and Wikesjö 2001). Long-term evaluations are probably linked to individual conditions such as changes in periodontal health status, toothbrushing habits and genetic and systemic conditions (Chambrone et al. 2010).

1.5 Adjunctive Use of Antibiotics in GTR Treatment

There are very few controlled trials assessing the need and the long-term efficacy of the adjunctive use of systemic antibiotics in periodontal regenerative surgical procedures (Demolon et al. 1993, 1994; Mombelli et al. 1996; Nowzari et al. 1995; Vest et al. 1999; Sculean et al. 2001; Loos et al. 2002), and the results are equivocal.

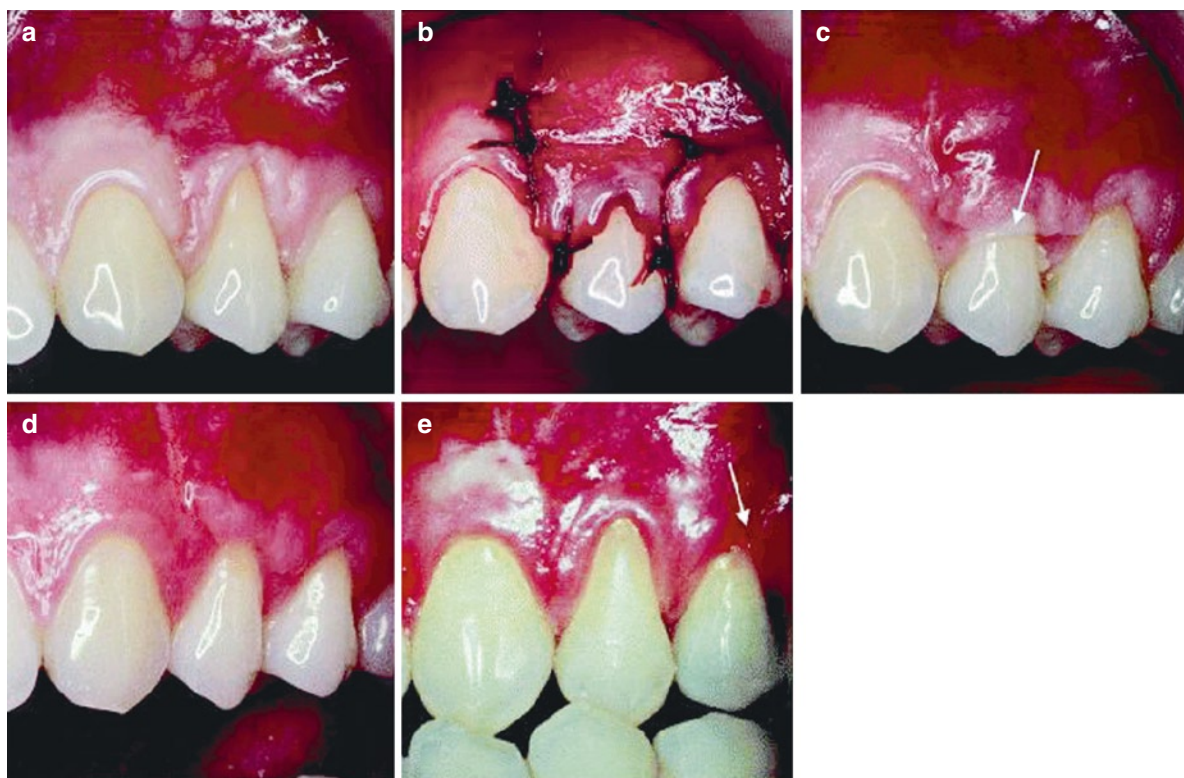


Fig. 1.10 (a) Preoperative view of buccal recession type defect on 24 in a smoker. (b) Surgical treatment of the recession on 24: The mobilized flap was coronally positioned over the membrane and anchored by an absorbable suture (membrane site). (c) Two weeks of healing following surgery on 24. A slight membrane exposure is visible (white arrow). (d) Six months postsurgical

view of 24. The root coverage is close to cemento-enamel junction. (e) Six years postsurgical view of 24. A relapse of the recession close to baseline level has occurred. Stillman's cleft on 25 indicates an ongoing traumatic buccal brushing technique (white arrow) (Leknes et al. 2005. Reprinted with permission from John Wiley & Sons)

1.6 Advantages and Disadvantages of the Use of GTR Treatment

Membrane exposure is a frequent complication of GTR treatment, with prevalence between 50% and 100% (Needleman et al. 2006; Stavropoulos et al. 2004a; Sanz et al. 2004; Eickholz et al. 2000; De Sanctis and Zucchelli 2000; Dörfer et al. 2000; Sculean et al. 1999a; Paolantonio et al. 1998; Christgau et al. 1997a; Falk et al. 1997; De Sanctis et al. 1996; Trombelli et al. 1995; Laurell et al. 1994) (Fig. 1.11). Machtei (2001) revealed that membranes that remained submerged during healing yielded better regenerative response compared to sites where the membrane became exposed. Mean horizontal attachment level gain for the submerged sites (3.72 ± 0.15 mm) was slightly greater than that of the exposed sites (3.06 ± 0.15 mm; $P=0.03$). For the intra-bony group, there were 309 sites in five studies; of these,

142 sites became exposed. Mean gain in vertical attachment level was 4.22 ± 0.15 mm and 4.69 ± 0.13 mm for the exposed and submerged group, respectively ($P=0.01$). Analyses of GTR in the management of gingival recession revealed that membrane exposure was negatively related with recession depth reduction (Danesh-Meyer and Wikesjö 2001).

Several putative pathogenic microorganisms were reported by Nowzari and Slots (1994, 1995) and Ling et al. (2003). They include *A. actinomycetemcomitans*, *P. gingivalis*, *B. forsythus* and *P. micros*. Yoshinari et al. (1998) indicated that numerous inflammatory cells adhered to and invaded the ePTFE membranes accompanied by bacterial contamination and that there was a tendency for a negative correlation between the increment number of bacteria and the gain of clinical attachment level. Since bacterial contamination appears to adversely affect the clinical outcome of regenerative procedures, it

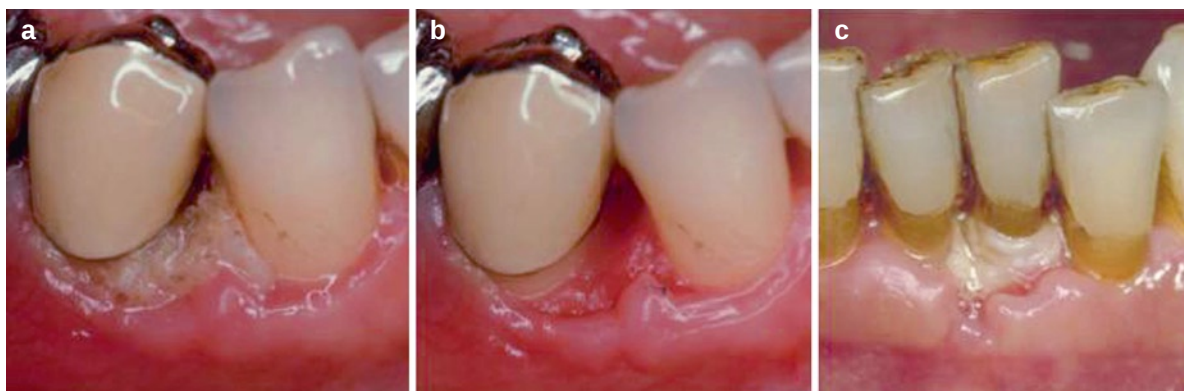


Fig. 1.11 (a) Postoperative exposure of a polydioxanone (PDS) membrane. (b) Defect space beneath PDS membrane is filled with fibrous-looking connective tissue. (c) Postoperative expo-

sure of a polylactic acid membrane (Christgau et al. 2002. Reprinted with permission from John Wiley & Sons)

seems logical to expect antimicrobial therapy to provide clinical benefits during procedures designed to treat osseous defects (Kornman and Robertson 2000). Depending on the degree of membrane exposure and on the extent of signs of infection near the exposed membrane, exposed membranes should be either removed or treated by a combination of additional systemic antibiotic therapy and topical application of 0.12% chlorhexidine digluconate (Villar and Cochran 2010).

Other postoperative complications, including bleeding, swelling, hematoma, erythema, suppuration, sloughing or perforation of the flap, membrane exfoliation and postoperative pain have been reported in the immediate postoperative period (Villar and Cochran 2010; Murphy 1995a, b).

1.7 GTR Barriers for Periodontal Regeneration

1.7.1 The Qualities of an “Optimal GTR Barrier” for Periodontal Regeneration

A wide range of membrane materials have been used in experimental and clinical studies to achieve GTR including polytetrafluoroethylene (PTFE), expanded PTFE (ePTFE), collagen, freeze-dried fascia lata, freeze-dried dura mater allografts, polyglactin, polylactic acid, polyglycolic acid, polyorthoester, polyurethane, polyhydroxybutyrate, calcium sulfate, microtitanium mesh and titanium foils (Hämmerle and Jung 2003).

Scantlebury (1993) presented design criteria for guided tissue regeneration (GTR) devices including biocompatibility, cell occlusion, space provision, tissue integration, and ease of use. Devices used for GBR in conjunction with endosseous implants should be safe and effective. Since no life-threatening diseases or deficiencies are treated, possible adverse effects emerging from the implanted devices should be kept to a minimum. At the same time, documentation of the effectiveness of the procedures and materials should be available (Hämmerle and Jung 2003). Certain critical criteria regarding membranes used for guided tissue regeneration have been postulated (Gottlow 1993; Hugoson et al. 1995; Hardwick et al. 1995; Hämmerle and Jung 2003):

- Safety requirements
- Highest possible biocompatibility
- Reliable cervical anchorage and sealing around the tooth
- Peripheral sealing and adjoining bone surfaces
- Space-making for selective tissue regeneration
- Barrier stability for clot protection
- Barrier tissue integration and prevention of barrier exposure
- Adapted barrier permeability
- Sufficient function time for periodontal regeneration to occur
- Adapted resorption pattern: tissue reactions resulting from the resorption of the membrane should be minimal, these reactions should be reversible, and they should not negatively influence regeneration of the desired tissues
- Clinical handling

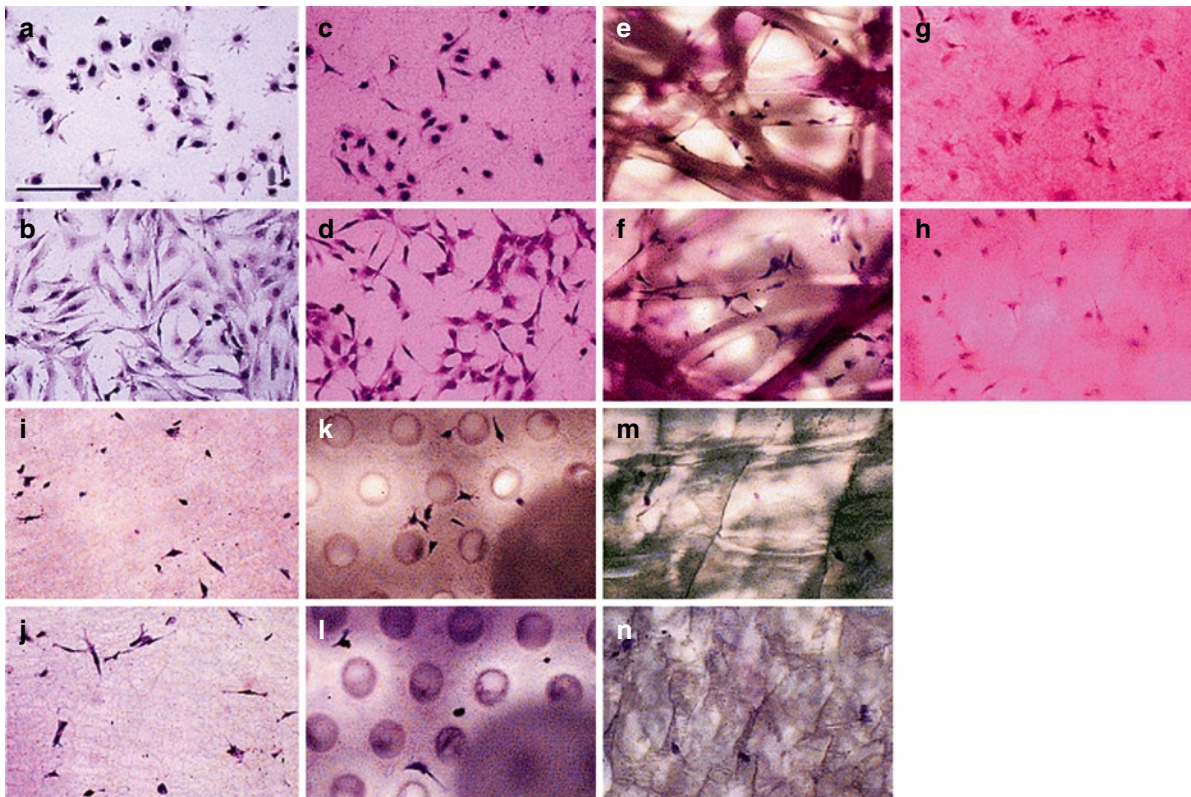


Fig. 1.12 Light microscope view of osteoblasts attached to various barriers at 1.5 and 24 h (Bar=200 μ m). Culture dish (CD) (a, b), Millipore filter (MP) (c, d), Resolut (RL) (e, f), BioMend (BM) (g, h), EpiGuide (EG) (i, j), Guidor (GD) (k, l),

Gore-Tex (GT) (m, n). Figures a, c, e, g, i, k and m are at 1.5 h and figures b, d, f, h, j, l and n are at 24 h (Wang et al. 2002. Reprinted with permission from John Wiley & Sons)

Ideally, barriers should facilitate cell attachment and coronal migration of the progenitor cells from the periodontium, since anchorage-dependent cells need to adhere to a substrata to be viable in promoting functions such as proliferation, migration, differentiation and maturation. Initial cell attachment to the barrier may help clot formation and wound stabilization. This earlier cell attachment can also act as a barrier stabilizer to minimize membrane micromovement and prevent the future disruption of new attachment formation (Takata et al. 2001a) (Figs. 1.12 and 1.13).

1.7.2 Nonresorbable Membranes

Nonabsorbable barriers were the first devices approved for clinical use. They maintain their structural integrity, and, consequently, the essential features they possess, for as long as they are left in the tissues. This

compositional and design stability provides the operator with complete control over time of application, with the potential to minimize variation in effectiveness (Tatakis et al. 1999).

Nonabsorbable barriers require, by their very nature, a second surgical procedure for removal. The need for additional surgery is accompanied by concerns over patient acceptance, time, cost, and possible morbidity associated with any surgical procedure. The function of the barrier membrane is temporary and, once the function is completed, there is no longer any need for it to remain in place. The tissue integration function of the membrane can be accomplished but is susceptible in this application to risk of latent or postsurgical bacterial contamination; together these indicate the removal of material to be in the best interest of the patient (Tatakis et al. 1999).

Besides expanded polytetrafluoroethylene devices, few other nonabsorbable materials have been investigated for use in guided tissue regeneration (Tatakis et al. 1999):

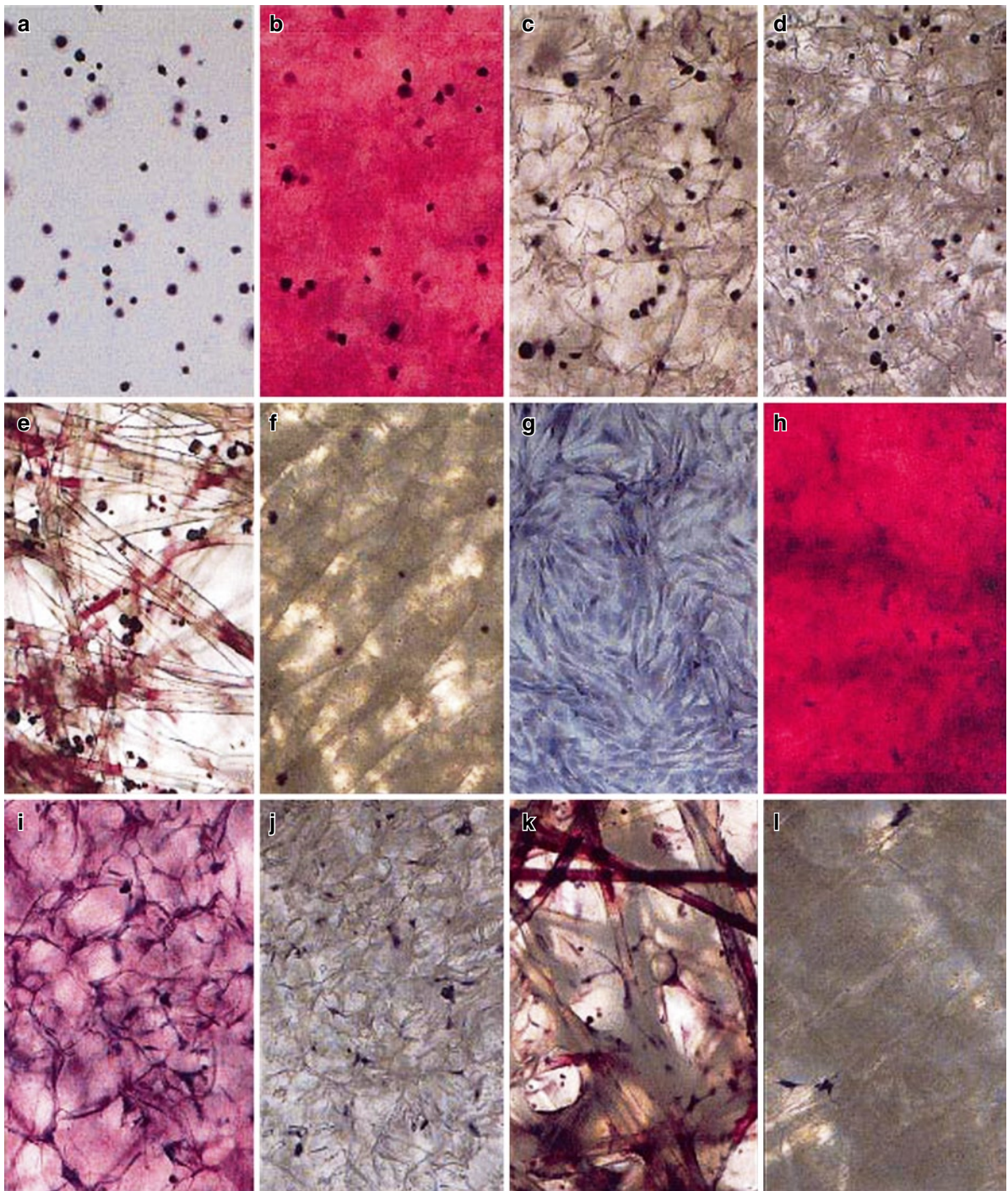


Fig. 1.13 Periodontal ligament (PDL) cells on various membranes at 1.5 h (top) and 5 days (bottom). (a, g), a plastic cover for cell culture slip (CD), (b, h), a bovine type I collagen membrane (BM), (c, i), a bovine type I atelocollagen membrane (TG), (d, j), a co-polymer of polylactic acid and polyglycolic acid membrane (RL), (e, k), a polylactic acid membrane (EG) and (f, l), an ePTFE membrane (GT). PDL cells were seen as rounded cells on the

membranes at 1.5 h after cell seeding (top). The initial number of PDL cells attached to the membrane was different among membranes. PDL cells were spindle or stellate shape and proliferated along the fibrous or porous structures of the membranes at day 5 (bottom). The rate of cell proliferation with time was different among the membrane examined. Hematoxylin staining 650 (Takata et al. 2001a. Reprinted with permission from John Wiley & Sons)

- Millipore filter
- Rubber dam – that offers little rigidity to assure space maintenance, can be tedious to manipulate, and due to the difficulty in completely covering the regenerated tissue may lead to important gingival recession (Cortellini and Prato 1994; Salama 1994; Paolantonio et al. 1998). It was showed that autoclave sterilization deteriorated the physical properties of rubber dams even though they seemed compatible to cultured human cells (Apinhasmit et al. 2003). When the connective tissue and bacterial deposits on rubber dam sheets and expanded PTFE membranes were compared similar results were found, suggesting that the healing process under both types of membranes was also comparable (Apinhasmit et al. 2002).
- Resin-ionomer barrier – that seems to have excellent space-making properties, but its tissue integration properties, if any, are unknown (Abitbol et al. 1995, 1996; Santi et al. 1997; Tatakis et al. 1999);
- Composite nonabsorbable devices made out of knitted nylon fabric mechanically bonded onto a semipermeable silicone membrane and coated with collagen peptides (BioBrane) appeared well tolerated by the tissues (Aukhil et al. 1986) but provided mixed results in terms of regenerative response (Tatakis et al. 1999). In class II furcation, at 4–6 weeks after membrane removal, no significant difference between results of treatment using the BioBrane membrane and those obtained using open debridement alone were observed, except in the measurement of horizontal open probing attachment, in which BioBrane-treated sites had a significant gain of soft tissue (Twohey et al. 1992).

1.7.2.1 Polytetrafluoroethylene

With the presentation of the first successful GBR procedures and the subsequent wide and successful application of ePTFE membranes, this material became a standard for bone regeneration. Expanded PTFE is characterized as a polymer with high stability in biological systems. It resists breakdown by host tissues and by microbes and does not elicit immunologic reactions (Hämmerle and Jung 2003).

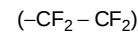


Fig. 1.14 Polytetrafluoroethylene polymer used in the manufacture of periodontal guided tissue regeneration devices

Polytetrafluoroethylene (dense-PTFE) is a fluorocarbon polymer with exceptional inertness and biocompatibility. Solid polytetrafluoroethylene is nonporous, does not allow tissue ingrowth and does not elicit a foreign-body reaction in tissue (Fig. 1.14). *Expanded polytetrafluoroethylene* (e-PTFE) is chemically identical to polytetrafluoroethylene, exhibits minimal inflammatory tissue reaction in a variety of tissue settings, when properly constructed allows tissue ingrowth, and has been used as a vascular graft material for over 20 years. Expanded polytetrafluoroethylene is polytetrafluoroethylene subjected to tensile stress during manufacture, resulting in differences in physical structure. It has a porous microstructure of solid nodes and fibrils. The size of the resulting fibrils and the spacing of the interconnecting nodes can be controlled through changes in the processing conditions. The optimal size of fibrils and internodal distance depends on the type of application the device is intended for (Tatakis et al. 1999) (Fig. 1.15).

Zellin and Linde (1996) investigated the influence of membrane porosity on the osteopromotive efficacy and to determine bone neogenesis times in a rat calvarian model. Three different ePTFE membrane qualities with different porosities (internodal distances <8, 20–25 and 100 μ m) were studied. The material with the smallest internodal distance did not integrate well with the surrounding soft tissue, leading to a lack of stabilization of the membrane and more soft tissue ingrowth from the side (Zellin and Linde 1996). The fact that different rates of osteogenesis were found indicates that sheer quoting of pore sizes (or internodal distances) is not completely adequate when discussing the effects of membranes on osteogenesis. One possible explanation is that the permeability to macromolecules and tissue fluid under physiological conditions is, in addition to the internodal distance, determined by other factors; one such factor might be fibrin gel formation within the membrane structure. Inflammatory cells occurring in the surrounding tissue, due to operative trauma, produce local growth factors that might enhance osteogenesis. Access of such factors, and transport of nutrients, to the site of osteogenesis might thus be hampered, especially

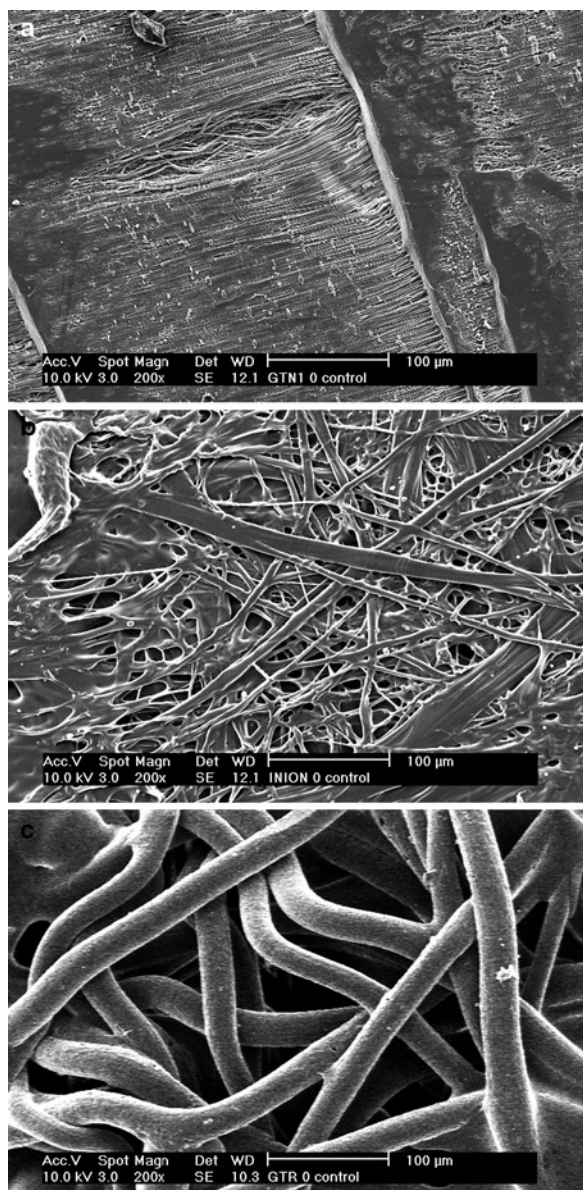


Fig. 1.15 Scanning electron micrographs of barrier membranes: (a) Gore-Tex (ePTFE)TM non-absorbable (GTN1); (b) Inion GTRTM bioabsorbable (INION); (c) Gore-Resolut XTTM bioabsorbable membrane (GTRX) (Chang et al. 2007. Reprinted with permission from John Wiley & Sons)

pronounced when membranes of smaller internodal distances are used (Zellin and Linde 1996). Wikesjö et al. (2003) evaluated the possibility of periodontal regeneration without gingival tissue occlusion.

Space-providing expanded polytetrafluoroethylene (ePTFE) membranes, with (macroporous) or without (occlusive) 300-µm laser-drilled pores, 0.8 mm apart, were implanted to provide for GTR in supra-alveolar periodontal defects created in dogs. It was concluded that tissue occlusion does not appear to be a critical determinant for GTR; substantial bone and cementum regeneration including a functionally oriented periodontal ligament occurs in the presence of space provision with or without tissue occlusion. However, tissue occlusion may be a requirement for optimal GTR (Wikesjö et al. 2003). However, remarkable clinical differences between the experimental conditions were noted. Whereas all sites receiving the porous membrane remained submerged for primary intention healing, 50% of sites receiving the occlusive membrane exhibited wound failure and membrane exposure. Obviously the porous membrane supported flap survival, probably being less of a challenge to the vascular support of the gingival flaps than the occlusive membrane (Polimeni et al. 2006). In a separate evaluation, Polimeni et al. (2005) investigated the effect of cell occlusion and space provision on alveolar bone regeneration in conjunction with GTR. Routine, critical-size, 6 mm, supra-alveolar, periodontal defects were created in dogs. Space-providing ePTFE devices, with or without 300-µm laser-drilled pores, were implanted to provide for GTR. It was reported that the relationship between space provision and bone regeneration was significant for both the porous and the occlusive GTR devices. Alveolar bone regeneration followed similar patterns in both groups. The authors speculated that the healing process supported by these two different devices may be similar or at least be similarly influenced by space provision. Nevertheless, the magnitude of newly formed bone was significantly increased for sites receiving occlusive GTR devices compared to sites receiving the porous devices when adjusted for the effect of the wound area. Thus, even if space provision appeared to be a critical factor for alveolar bone regeneration, device occlusivity appeared to provide adjunctive effects (Polimeni et al. 2005).

In clinical studies, treatment of vertical osseous or furcation defects with nonporous (TefGen, GD Inc.,

and Sacramento, CA) or porous (Gore-Tex, Gore and associates Inc., Flagstaff, AZ) polytetrafluoroethylene membranes in combination with a xenograft or with demineralized freeze dried bone allografts resulted in statistically significant improvement in open and closed probing measurements, with no significant difference between treatment groups (Walters et al. 2003; Lamb et al. 2001).

The Gore-Tex expanded polytetrafluoroethylene periodontal device features two structural designs to address specific needs:

- An open microstructure collar, corresponding to the coronal aspect of the device, to promote connective tissue ingrowth, and to support wound stability and inhibit epithelial apical migration. This part of the device is 1.0-mm thick, is of low-density (0.2 g/mL) and 90% porous (100–300 μ m between nodes) expanded polytetrafluoroethylene.
- The remainder of the device is a partially occlusive, structurally relatively stable membrane serving to provide a space for regeneration as well as a barrier toward gingival flap tissue invasion or collapse onto the root surface. It consists of 0.15-mm thick, higher-density (1.5 g/mL) and 30% porous (<8 μ m between nodes) expanded polytetrafluoroethylene (Tatakis et al. 1999).

The efficacy of expanded polytetrafluoroethylene barriers in guided tissue regeneration of intrabony defects has been evaluated by several investigators (Caffesee et al. 1997; Cortellini et al. 1995a; Kilic et al. 1997; Kim et al. 2002; Paolantonio et al. 1998; Silvestri et al. 2000, 2003; Tonetti et al. 1996, Yoshinari et al. 2001; Zucchelli et al. 1999, 2002; Klein et al. 2001) (Fig. 1.16).

The use of expanded polytetrafluoroethylene devices has been associated with minor complications such as pain, purulence, swelling and tissue sloughing, with an incidence slightly higher than that reported for conventional periodontal surgery (Tatakis et al. 1999).

Many of the factors critical for successful bone formation were identified in experimental studies applying ePTFE membranes. Furthermore, clinical protocols regarding surgical procedures, postoperative care and the healing times required were

established using nonresorbable membranes. Today, as evidence of the effectiveness of bioresorbable membranes increases, nonresorbable membranes are losing importance in clinical practice and their use is increasingly limited to specific indications. Since the use of ePTFE membranes has been documented to result in successful GBR therapy, results obtained using new materials should always be compared with results of ePTFE membranes (Hämmerle and Jung 2003).

1.7.2.2 Titanium-Reinforced ePTFE

It has been showed that space provision is a critical factor for periodontal regeneration including alveolar bone, cementum and a functionally oriented periodontal attachment (Haney et al. 1993; Sigurdsson et al. 1994, 1995; Trombelli et al. 1999; Polimeni et al. 2004a, b, c, 2005) (Fig. 1.17). Also periodontal regeneration seems to be compromised in the absence of space provision such as following gingival flap surgery alone when the gingival flaps collapse onto the root surface or following GTR when the GTR device has collapsed or been compressed onto the root surface (Haney et al. 1993; Sigurdsson et al. 1994).

In situations where bone formation is desired in large defects or in supracrestal areas, conventional ePTFE membranes do not adequately maintain space unless supported by grafting materials. The alternative approach involves the use of membranes with a stable form, such as titanium-reinforced membranes (Fig. 1.18). Titanium-reinforced membranes consist of a double layer of ePTFE with a titanium framework interposed. The rigidity of the reinforced expanded polytetrafluoroethylene device supports improved space provision and maintenance. Recent research has demonstrated the successful use of these membranes in vertical ridge augmentation, in the treatment of large defects in the alveolar process, intrabony defects and in gingival recessions (Cortellini and Tonetti 2005; Hämmerle and Jung 2003; Zucchelli et al. 2002; Cortellini et al. 1995a; Tinti and Vincenzi 1994).



Fig. 1.16 Combined facial-distal intrabony and facial class II furcation defects managed with combined regenerative therapy with expanded polytetrafluoroethylene. **(a)** Facial view (mirror) tooth number 47 at surgical exposure and debridement. **(b)** Re-entry at 27 months for second-stage enhancement of residual pocket/osseous defect distal tooth number 46 revealing complete furcal and intrabony defect bone fill of former defects on tooth number 47. **(c)** Probe in root trunk concavity without

horizontal penetration (that is, bone-to-facial aspect of furcation fundus). **(d)** Preoperative radiograph depicting multiple osseous defects. **(e)** Radiograph 18 months after surgery depicting bone apposition in tooth numbers 46 and 47 areas with residual distal angular osseous defects in tooth numbers 46 and 47 (McClain and Schallhorn 2000. Reprinted with permission from John Wiley & Sons)

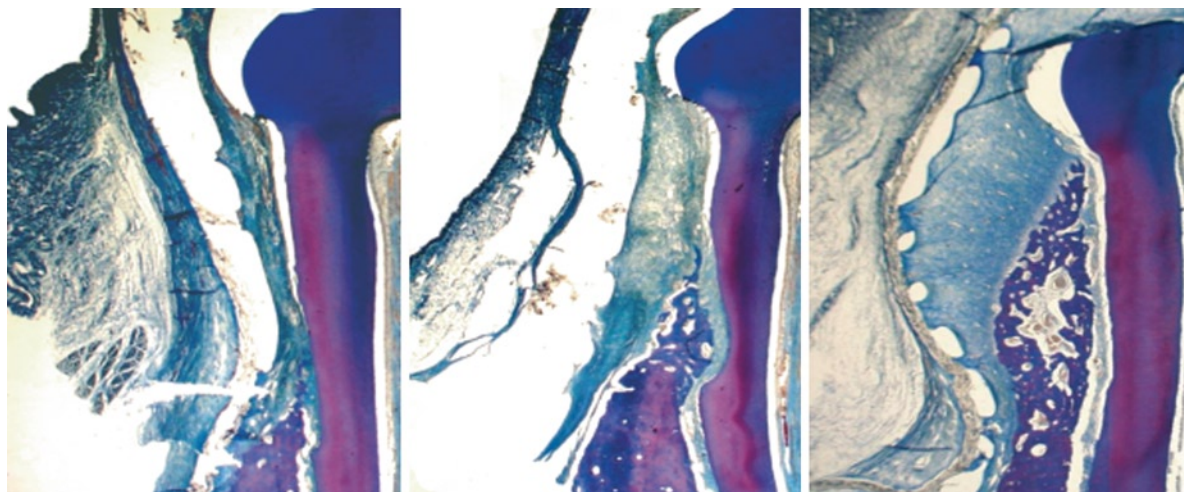


Fig. 1.17 Representative photomicrographs of supra-alveolar periodontal defects with space-providing occlusive ePTFE devices. The effect of space provision can be observed in three different sites. Sites providing a small wound area resulted in

limited bone formation (*left and center*). Sites providing a larger wound area resulted in enhanced bone formation (*right*) (Polimeni et al. 2004b. Reprinted with permission from John Wiley & Sons)

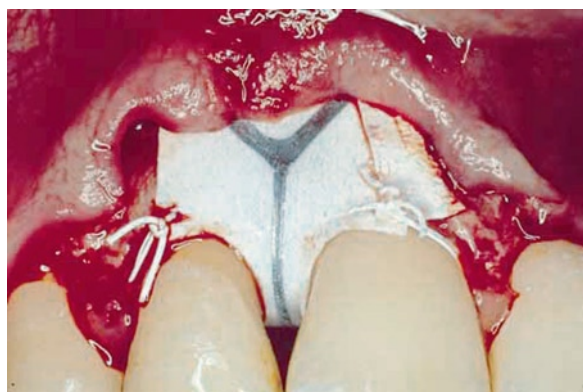


Fig. 1.18 Modified papilla preservation technique. A titanium-reinforced barrier membrane positioned to isolate the defect (buccal view) (Cortellini and Tonetti 2000. Reprinted with permission from John Wiley & Sons)

1.7.3 Bioresorbable Membranes

Toward the end of the 1980s, and after the surfacing of the concept of biodegradation, the second generation resorbable barriers were introduced. They were intended to omit the surgery of membrane retrieval and reduce the treatment time (El Helow and El Askary 2008). They include collagens such as:

- Colética (Colética, Lyon, France)
- Bio-Gide (Geistlich Biomaterials, Wolhusen, Switzerland)
- Bio-mend (cross-linked bovine type I collagen)
- Polyglactin 910 knitted mesh such as Vicryl (Ethicon, Norderstedt, Germany)
- Polylactic acid such as Atrisorb (DL-lactide polymer, Atrix Laboratories Inc., Fort Collins, CO); 37% polylactic acid, 63% pyrrolidine
- Polyglycolic acid
- Copolymer of polylactic and polyglycolic acid such as Resolut XT (Gore and associates Inc., Flagstaff, AZ)
- Freeze-dried fascia lata
- Laminar bone barrier (Lambone, Pacific Coast Tissue Bank, Los Angeles, CA) made from 100- to 300- μ m thick sheets of demineralized, freeze-dried ethylene oxide sterilized cortical bone
- Polyhydroxybutyrate (PHB)
- PHB copolymerized with hydroxyvalerate
- PHB copolymerized with hydroxyvalerate and polyglactin 910

GTR membrane materials, per se, may influence cell proliferation in the process of periodontal tissue/bone regeneration. It has been showed that the bioabsorbable membranes were more suitable to stimulate

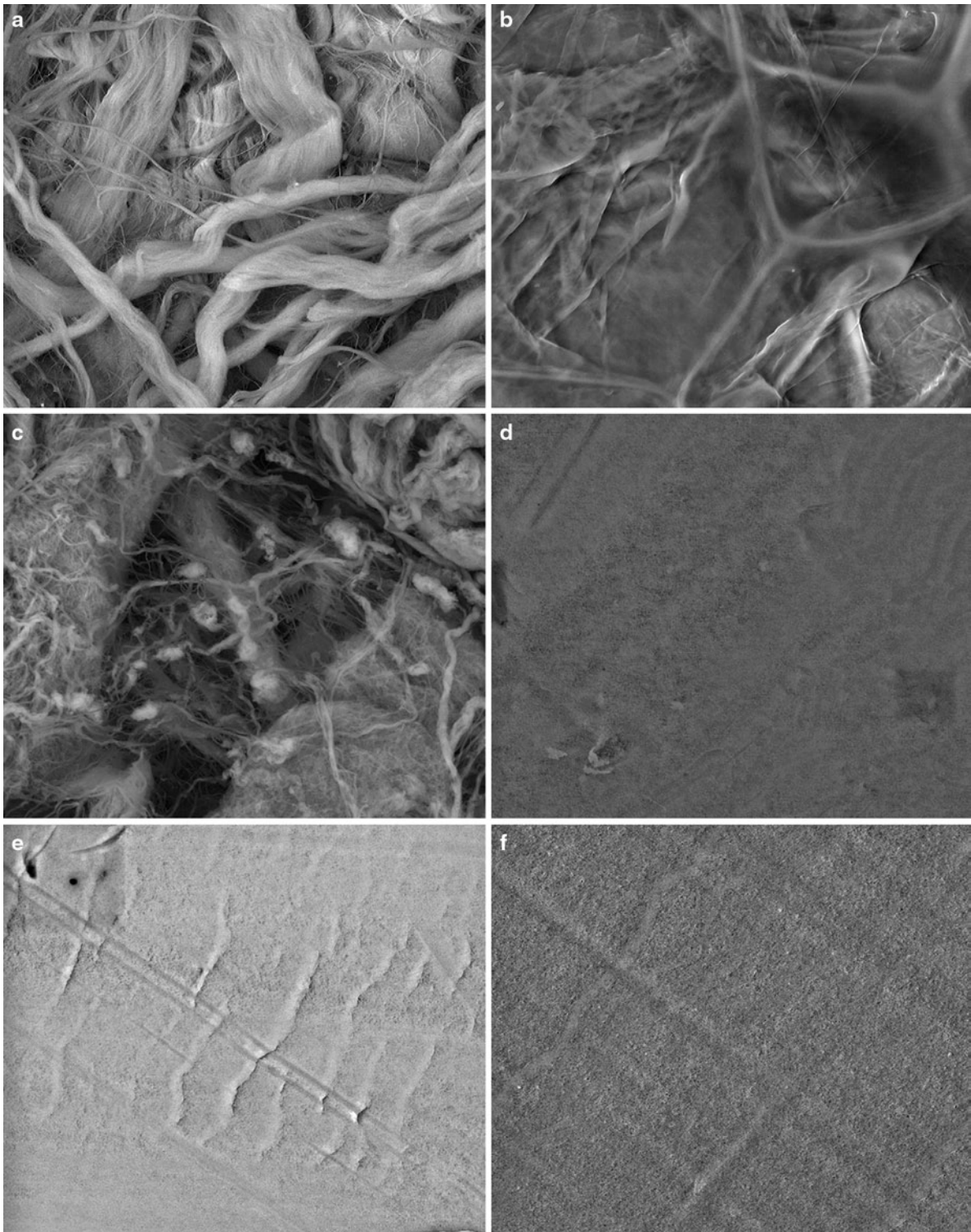


Fig. 1.19 Surface SEM micrographs of the examined bioabsorbable and nonresorbable membranes: **(a)** TutoDent® (bovine type I collagen; Tutogen medical GmbH, Neunkirchen, Germany), **(b)** Resodont® (equine type I collagen; Resorba®, Nurnberg, Germany), **(c)** Bio-Gide® (porcine type I and III collagen; Geistlich Biomaterials, Wolhusen, Switzerland), **(d)** TefGen-FD® (nano-porous polytetrafluoroethylene (n-PTFE);

Lifecore Biomedical GmbH, Alfter, Germany), **(e)** Cytoplast® Regentex GBR-200 (CT) (high-density polytetrafluoroethylene (d-PTFE); Oraltronic® Dental Implant Technology GmbH, Bremen Germany) and **(f)** ACE (non-textured polytetrafluoroethylene (PTFE); ACE Surgical Supply Co., Brockton, USA) (Kasaj et al. 2008. Reprinted with permission from BioMend Central)

Table 1.5 Characteristics of bioresorbable membranes

<i>Advantages</i>
• No need for membrane removal surgery • Simplified surgical procedure with an implant system with two-stage surgical approach • Wider range of surgical techniques possible at abutment connection (which coincides with membrane removal for nonresorbable membranes) • Better cost-effectiveness • Decreased patient morbidity
<i>Disadvantages</i>
• Uncontrolled duration of barrier function • Resorption process possibly interfering with wound healing and bone regeneration • Need for membrane supporting material

Source: Hämmerle and Jung (2003). Reprinted with permission from John Wiley & Sons

cellular proliferation compared to nonresorbable PTFE membranes (Kasaj et al. 2008) (Fig. 1.19).

Apart from the fact that the surgical intervention for removal of the membrane is omitted, bioresorbable membranes offer some additional advantages (Hämmerle and Jung 2003) (Table 1.5).

Several disadvantages have also been revealed. By their inherent nature, absorbable barriers offer *limited control over the length of application*. This is because the disintegration process starts upon placement in the tissues, and the ability of each individual patient to degrade a particular biomaterial may vary significantly, particularly for materials requiring enzymatic degradation (such as collagen). Absorbable devices should maintain their in vivo structure for at least 4 weeks, but because of their biodegradability, absorbable devices elicit inevitable and necessary tissue reactions that may influence wound healing. Ideally, these inflammatory reactions should not compromise the intended regenerative outcome. This probably requires that such reactions be of limited magnitude and tolerable nature and occur after the critical early healing sequence (Hämmerle and Jung 2003).

Bioresorbable membranes that are commercially available at present *are not capable of maintaining adequate space* unless the defect morphology is very favorable. Even if the membranes initially seem able to maintain space, they generally lose their mechanical strength soon after implantation into the tissues. Only in situations where the bony borders of the defects adequately support the membrane have favorable results been reported. When defects are not

space-making by themselves, failure of bone regeneration results. Therefore, they need to be supported in one way or another. Biomaterials without their own space-maintaining capacity have limited relevance for GTR as they collapse into the defect area obstructing the native potential for regeneration. Although use of other materials (bone derivatives or substitutes) has been suggested to support space maintenance, such biomaterials may ultimately compromise periodontal wound healing and regeneration, including alveolar bone (Danesh-Meyer and Wikesjö 2001; Hämmerle and Jung 2003).

The clinician must also consider that bioresorbable membranes inevitably evoke *inflammatory processes in their degradation process*, which will influence wound healing and ultimately their clinical relevance (Danesh-Meyer and Wikesjö 2001).

Resorbable periodontal membranes are subjected to proteolytic degradation (Figs. 1.20 and 1.21). The ester-composed synthetic polymers of polylactic–citric acid–ester barriers (PLA) and glycolide–lactic copolymer membranes (PGL) are degraded in vivo by enzymatic hydrolysis. Proteolytic enzymes released from polymorphonuclear leukocytes, especially those with an esterase activity, participate in the biodegradation process. As a result of membrane placement, an inflammatory process is initiated that is associated with a release of lysosomal enzymes from activated polymorphonuclear leukocytes accumulating at the GTR-treated periodontium (Buchmann et al. 2001).

Absorbable materials used for guided tissue regeneration devices fall into two broad categories: natural products and synthetic materials.

1.7.3.1 Natural Products

Collagen has been used extensively for the manufacture of biomedical devices because of its biologic and physical properties, and ample availability. Collagen forms a 3-D cellular matrix of all tissues. Collagen surrounds the cells and gives each tissue its characteristic structure, texture and shape. It is the structural building block of the body. In tendons, collagen has a strength equal to light steel wire. Collagen in the cornea is transparent, in heart valves it is fatigue resistant and in renal glomeruli it provides an excellent filtration system (Khor 1997; O’Grady and Bordon 2003).

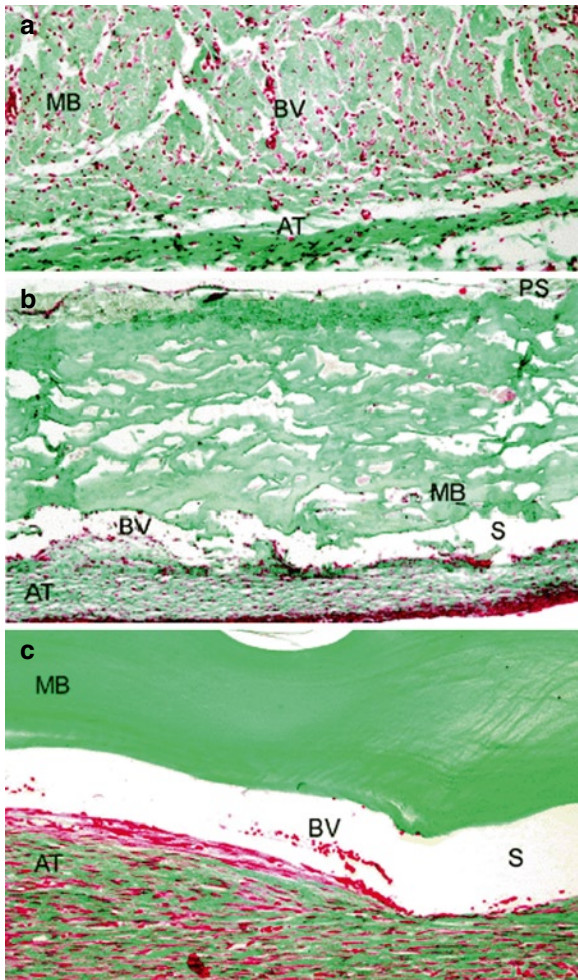


Fig. 1.20 (a) Complete vascularization of Bio-Gide® after 2 weeks. Note the interconnective porous system like structure (original magnification $\times 40$). Goldner trichrome stain. (b) Stratified appearance of BioMend® with large interstices. Two weeks following implantation, some blood vessels started to invade the slit separating the membrane from the adjacent connective tissue (original magnification $\times 40$). (c) Two weeks following implantation, Ossix® was clearly separated from the adjacent connective tissue by a split (original magnification $\times 40$). AT adjacent tissue, BV blood vessels, MB membrane body, PS polycarbonate spacer, S split (Rothamel et al. 2005. Reprinted with permission from John Wiley & Sons)

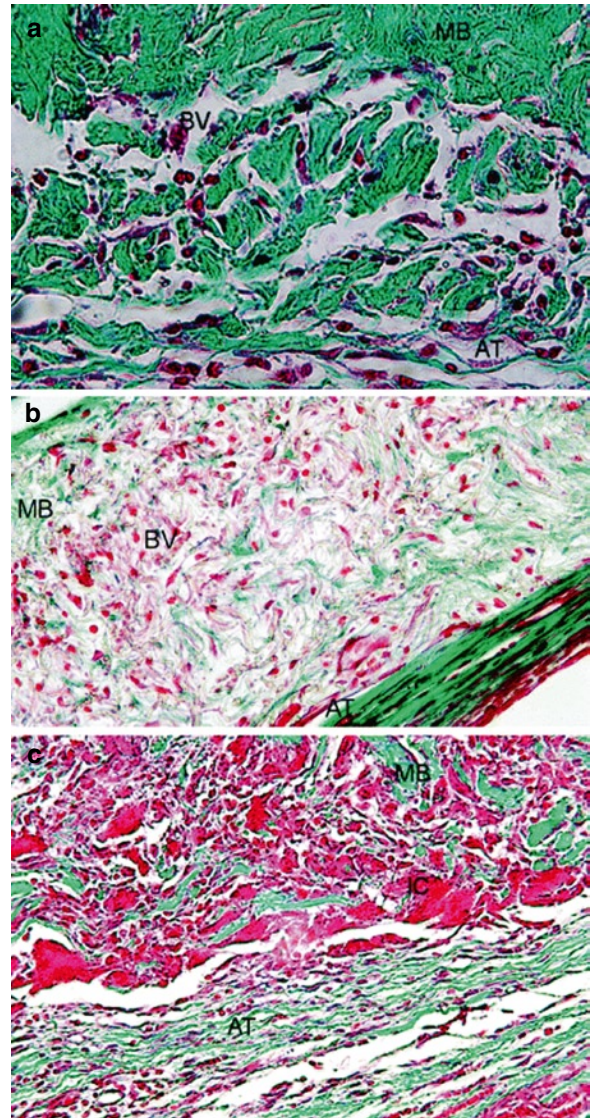


Fig. 1.21 (a) Nearly complete vascularization of TutoDent® after 4 weeks. Note the interconnective porous system like structure (original magnification $\times 200$). (b) Entire organization and biodegradation of Bio-Gide® 4 weeks following implantation (original magnification $\times 40$). (c) Infiltration of inflammatory cells in the connective tissue adjacent to the external surface of VN(3) was observed after 4 weeks (original magnification $\times 200$). AT adjacent tissue, BV blood vessels, IC inflammatory cells, MB membrane body (Rothamel et al. 2005. Reprinted with permission from John Wiley & Sons)

There are over 20 known types of collagen (Table 1.6). The fibril-forming (fibrillar) collagens include collagen type I [$\alpha 1(I)$]₂ $\alpha 2(I)$ comprising fibril bone, skin, tendons, ligaments, cornea and internal organs, accounting for 90% of body collagen; collagen

type II [$\alpha 1(II)$]₃ comprising fibril cartilage, intervertebral disc, notochord and vitreous humor of the eye; collagen type III [$\alpha 1(III)$]₃ comprising fibril skin, blood vessels and internal organs; collagen type V [$\alpha 1(V)$]₂ $\alpha 2(V)$ and $\alpha 1(V)$ $\alpha 2(V)$ $\alpha 3(V)$ fibril (with type I)

Table 1.6 List of some of the collagen types and information on chain composition, structure, tissue location and related information

Types	Chain composition	Structural details	Localization	Notes
I	$[\alpha 1(I)]_2[\alpha 2(I)]$	300 nm, 67-nm banded fibrils	Skin, tendon, bone, etc.	90% of all collagen of the human body. Scar tissue the end product when tissue heals by repair
II	$[\alpha 1(II)]_3$	300 nm, small 67-nm fibrils	Cartilage, vitreous humor	Articular cartilage
III	$[\alpha 1(III)]_3$	300 nm, small 67-nm fibrils	Skin, muscle, frequently with type I	Collagen of granulation tissue, and is produced quickly by young fibroblasts before the tougher type I collagen is synthesized
IV	$[\alpha 1(IV)]_2[\alpha 2(IV)]$	390 nm C-term globular domain, nonfibrillar	All basal lamina	Basal lamina
V	$[\alpha 1(V)][\alpha 2(V)][\alpha 3(V)]$	390 nm N-term globular domain, small fibers	Most interstitial tissue, assoc. with type I	Most interstitial tissue, assoc. with type I
VI	$[\alpha 1(VI)][\alpha 2(VI)][\alpha 3(VI)]$	150 nm, N1C term. Globular domains, microfibrils, 100-nm banded fibrils	Most interstitial tissue, assoc. with type I	Most interstitial tissue, assoc. with type I
VII	$[\alpha 1(VII)]_3$	450 nm, dimer	Epithelia	Epithelia
VIII	$[\alpha 1(VIII)]_3$	130 nm, N1C term. Globular domains	Some endothelial cells	Some endothelial cells
IX	$[\alpha 1(IX)][\alpha 2(IX)][\alpha 3(IX)]$	200 nm, N-term. Globular domain, bound proteoglycan	Cartilage, assoc. with type II	Cartilage, assoc. with type II
X	$[\alpha_1(X)]_3$	150 nm, C-term. Globular domain	Hypertrophic and mineralizing cartilage	Hypertrophic and mineralizing cartilage
XI	$[\alpha 1(XI)][\alpha 2(XI)][\alpha 3(XI)]$	300 nm, small fibers	Cartilage	Cartilage
XII	$\alpha 1(XII)$	75-nm triple helical tail, central globule, three 60-nm globule arms	Interacts with types I and III	Interacts with types I and III
Mainly types I–IV have been utilized to varying degrees in tissue-engineering related biomaterials studies, with some efforts on the other types shown.				

Source: Abraham et al. (2008). Reprinted with permission from John Wiley & Sons

comprising tissue similar to those for type I collagen; and collagen type XI $\alpha 1(XI)$ $\alpha 2(IX)$ $\alpha 3(XI)$ fibril (with type II) comprising tissue similar to collagen type II. For all collagen types, each collagen chain of <1,000 amino acids is composed of three left-handed α -helix chains that twist together to form the right-handed helix of the collagen molecule. The collagen molecule is about 300 kDa, composed of <10% each of proline and hydroxyproline, and has glycine present at every

third amino acid position (Abraham et al. 2008; O'Grady and Bordon 2003).

The subunit chains of collagen are synthesized from free amino acids, mostly in fibroblasts and osteoblasts, by the ribosomes of the rough endoplasmic reticulum as larger pro-chains. Proline and lysine residues are selectively hydroxylated enzymatically, and the pro-chains are often glycosylated during this stage. The primary structure of this protein is unique, showing a

strong sequence homology across genus and adjacent family lines. The primary structure (with its high content of proline and hydroxyproline, and with every third amino acid being glycine) contributes to forcing each collagen subunit into a helical structure. The three subunits are then arranged in the form of the triple helical procollagen molecule, similar to a triple-stranded rope, which is then released into the extracellular space. The pro-collagen is then transformed in the extracellular space by specific peptidases to form the collagen monomer. In addition to the helical portion of the molecule, the terminal amino acid sequence at each end of the molecule is comprised of short (less than 5% of the total) nonhelical domains called telopeptides, which are involved in polymerization by noncovalent binding to sites on adjacent helices (Khor 1997; O'Grady and Bordon 2003).

Collagen used for medical devices is derived from several animal sources including bovine skin, tendon, intestine or sheep intestine. Isolation and purification follows one of two ways. The first is enzymatic preparation of soluble collagen and the other is chemical extraction of fibrillar collagen from collagenous tissue. Following isolation and purification, collagen is processed by several means to manufacture gels, sponges, filaments, membranes, etc. (Tatakis et al. 1999). Collagenous tissues obtained from the abattoir, cadaver or patient begin to degrade immediately. Therefore, in the exploitation of tissue as clinical material this deterioration must be arrested and deferred, preferably beyond the recipient's natural life. The aim is to prolong the materials' original structural and mechanical integrity and remove or at least neutralize the antigenic properties attributed to these materials. Methods typically concentrate on creating new additional chemical bonds between the collagen molecules. These supplementary links reinforce the tissue to give a tough and strong but nonviable material that maintains the original shape of the tissue (Khor 1997).

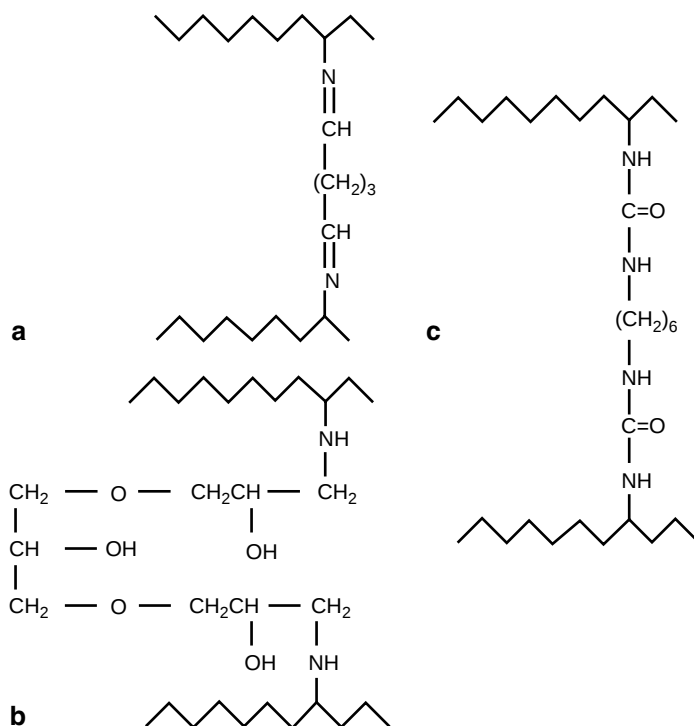
Collagen can be prepared from a number of sources using a variety of techniques. The resulting membranes generally are formed by reconstitution. In this process, collagen derived from a rich source such as skin dermis or tendon is isolated and purified, then precipitated into fibrillar form by changing the ionic strength, pH, or by elevating the temperature to 37°C followed by air evaporation and freeze drying (Patino et al. 2002; Bell et al. 1979). Collagen may be further treated with

pepsin for removal of the terminal telopeptides of the molecule, which is the major inflammatory component (Patino et al. 2002; Blumenthal 1993).

Physical and chemical methods for the treatment of collagenous tissue are available. The process of stabilizing tissue involves the chemical agent or the physical process initiating, ideally, irreversible and stable intra- and intermolecular chemical bonds between collagen molecules. Preferably, the agent promotes bonds between the functional groups of the amino acids. Physical methods include drying, heating or exposure to ultraviolet or gamma radiation. Unlike *chemical cross-linking*, these methods do not introduce toxic chemicals into the tissue, but this does not preclude undefined side-effects that may arise due to these processes. The predominant chemical agents that have been investigated for the treatment of collagenous tissue for bioprotheses are *glutaraldehyde*, *formaldehyde*, *polyepoxy compounds*, *acyl azide*, *carbodiimides* and *hexamethylene diisocyanate* (Fig. 1.22). These treatments give a biomaterial that is nonviable, the intended advantage being resistance to in vivo degradation (Khor 1997). Glutaraldehyde (GA) is the most widely used chemical cross-linking agent because it stabilizes collagen efficiently. However, GA-cross-linked biomaterials are poorly biocompatible with some cell lines including human fibroblasts, osteoblasts, Chang cells and endothelial cells. The side effects of GA treatment are, at least in part, attributed to the degradation of the GA-derived cross-links and to the continual release of aldehydes that contribute to prolonged toxic effects. To overcome these disadvantages, alternative cross-linking with diphenylphosphoryl azide (DPPA) was developed. Cross-linking with DPPA increases resistance to degradation of collagen biomaterial and seems to be more biocompatible than cross-linking with GA (Marinucci et al. 2003 and references therein).

Collagen membranes are resorbed by the enzymatic activity (collagenase) of infiltrating macrophages and polymorphonuclear leukocytes (Tatakis et al. 1999) and resorption velocity can vary greatly, depending on collagen source and modifications. The enzyme collagenase initiates membrane resorption at the specific site. Resulting fragments denature and become gelatine, which is then degraded to amino acids by gelatinases and other enzymes. Some periodontal pathogens like *Porphyromonas gingivalis* produce collagenase. Since bacteria colonize the exposed membrane during healing, uncontrolled degradation can take place

Fig. 1.22 Simplified representation of cross-linking for glutaraldehyde (a) polyepoxy compounds, (b) hexamethylene diisocyanate (c) to collagen (Modified from Khor 1997. Reprinted with permission from Elsevier)



resulting in unfavorable outcome (Sela et al. 2003; Aurer and Jorgic-Srdjak 2005). It has been shown that the degradation of cross-linked collagen membranes was significantly slower compared with non-cross-linked membranes (Pitaru et al. 1988; Paul et al. 1992; Brunel et al. 1996) and that the resorption rate depends upon the degree of cross-linking (i.e., the higher the degree of cross-linking, the longer the resorption rate) (Brunel et al. 1996). The influence of different cross-linking techniques on biodegradation over time, foreign body reactions, tissue integration and vascularization has recently been examined in a histomorphometric study in rats (Rothamel et al. 2005). It was observed that cross-linking of bovine and porcine-derived collagen types I and III seemed to be associated on the one hand with prolonged biodegradation, but on the other hand with foreign body reactions and decreased tissue integration and vascularization (Rothamel et al. 2005). Marinucci et al. (2003) showed that the diphenylphosphoryl azide method is preferable to the glutaraldehyde method and that membranes made of collagen and chondroitin sulfate are more biocompatible than membranes made of pure collagen. When the biocompatibility of differently cross-linked collagen membranes in cultures of human PDL fibroblasts and

human osteoblast-like cells (SaOs-2) were evaluated, different effects were revealed: Bio-Gide® (BG) (Geistlich Biomaterials, Wolhusen, Switzerland) (non-crosslinked porcine types I and III collagen), TutoDent® (TD) (Tutogen, Carlsbad, CA, USA) (non-crosslinked bovine type I collagen) and Ossix® (OS) (3i, Colbar R&D Ltd, Ramat Husharon, Israel) (enzymatic-cross-linked bovine type I collagen) promoted, while, in contrast, BioMend® (BM) (Sulzer Medica, Colla-Tec, Inc., Plainsboro, NJ, USA) (glutaraldehyde cross-linked bovine type I collagen) inhibited the attachment and proliferation of human PDL fibroblasts and human SaOs-2 osteoblasts (Rothamel et al. 2004). Similar results were reported by Sela et al. (2009) who showed that while all collagen membranes are prone to lysis by oral bacterial proteases, cross-linked membranes are more resistant to proteolysis. Furthermore, therapeutic concentrations of the antibacterial and antibiotic agents chlorhexidine, cetylpyridiniumchloride, minocycline and doxycycline were found to partially inhibit the enzymatic breakdown of the membranes, while metronidazole had no such effect. These results suggest that the presence of *P. gingivalis* cells, extracellular vesicles and enzymes in the vicinity of regeneration

membranes in the periodontium may change their physical structure and therefore alter their biological properties. Furthermore, the use of cross-linked collagen membranes and antibacterial agents may significantly inhibit this proteolytic process (Sela et al. 2009).

It should be noted that a prolonged resorption rate of the collagen membrane does not always result in greater periodontal/bone regeneration (Bunyaratavej and Wang 2001). In a dog model, Crigger et al. (1996) evaluated healing following treatment of periodontal defects using two collagen barrier membranes with different degrees of cross-linking, and compared the results to those following use of an expanded polytetrafluoroethylene (ePTFE) membrane. Clinical observations indicated that the highly cross-linked, slow-resorbing collagen membrane did not integrate with the tissues the way the less cross-linked, rapid-resorbing collagen did. Membrane exposure was typical for the slow-resorbing membrane in contrast to the rapid-resorbing membrane which remained covered. The inferiority of the slow-resorbing membrane was evident by the extensive clinical recession and the attachment level measurements taken at 6 months, and it was decided to omit this membrane from histometric analysis. Histological examination of root surfaces treated with rapid-resorbing collagen or ePTFE membranes revealed substantial reparative healing. The connective tissue repair amounted to 84% of the treated root surface height for the rapid-resorbing collagen and 53% for the ePTFE membrane (difference not statistically significant). However, the connective tissue repair to the rapid-resorbing collagen group root surfaces was often associated with a layer of ankylosis (44% vs. 8% of the ePTFE group). It was concluded that the rapid-resorbing collagen membranes and the ePTFE membranes seem capable of stimulating periodontal connective tissue repair, whereas the slow-resorbing collagen membranes were unsuccessful in this effort.

The properties of a GTR or GBR membrane may be affected, in addition to the effects of cross-linking, also by the surface topography of a membrane (Brunette 1988) and the origin of the collagen used (Takata et al. 2001a, b; Wang et al. 2002; Behring et al. 2008).

Collagen also possesses a chemotactic function for fibroblasts that aids in cell migration to promote primary wound closure (Postlethwaite et al. 1978). Alpar

et al. (2000) indicated that the biodegradable collagen membrane exhibited excellent cytocompatibility (Figs. 1.23–1.25). No changes in the periodontal ligament fibroblasts and human osteoblast-like cells were found in fibroblast and osteoblast-like cell cultures. On the contrary, the ePTFE and polylactic acid membranes induced slight to moderate cytotoxic reactions which may reduce cellular adhesion. Thus, gap formation between the barrier surface and the connective tissue may be promoted which may facilitate epithelial down-growth and microbial accumulation. Consequently, these effects may reduce the potential gain in periodontal attachment (Alpar et al. 2000).

Many different collagen barrier devices are on the market or under development today (Behring et al. 2008) (Table 1.7).

Bio-Gide® collagen membrane (Geistlich Biomaterials, Wolhusen, Switzerland) is derived from porcine dermis and is composed of type I and III collagen without further cross-linking or chemical treatment. Fixation by sutures or pins is possible. The coherent collagen fibers swell and form a unified basic tissue structure. As a result, adaption to the bone wall and complete closure of the bony defects are easily achieved. Because of its elasticity, Bio-Gide® has to be used in combination with space-making bone graft materials, e.g., autogenous bone, bone substitutes (Bio-Oss®, etc.). The Bio-Gide® membrane is a bilayer membrane (Fig. 1.26). The dense superior membrane layer, which faces the soft tissue, is cell occlusive and prevents invasion of soft connective tissue cells into the membrane-protected space. The porous inferior layer, which faces the bony defect, consists of loosely arranged collagen fibers which act to stabilize the clot and enable bone cells to become integrated into the membrane. The dense cell-occlusive layer is a barrier to soft tissue ingrowth and serves as a soft tissue scaffold. Granulation cells adhere to the natural collagen surface. The porous side of the membrane is an open-pore, three-dimensional collagen matrix, which promotes cell integration (<http://www.osteohhealth.com/Bio-Gide.html>).

Bio-Gide® was extensively used in guided bone regeneration (Lang et al. 2007; Hämmerle and Lang 2001; Hämmerle and Jung 2003; Juodzbalsys et al. 2003; Zitzmann et al. 1997, 2001; Hämmerle and Lang 2001), healing of intrabony peri-implantitis (Schwarz et al. 2006a; Tawil et al. 2001) (Fig. 1.27), for the treatment of implant dehiscence defects (Oh et al. 2003;

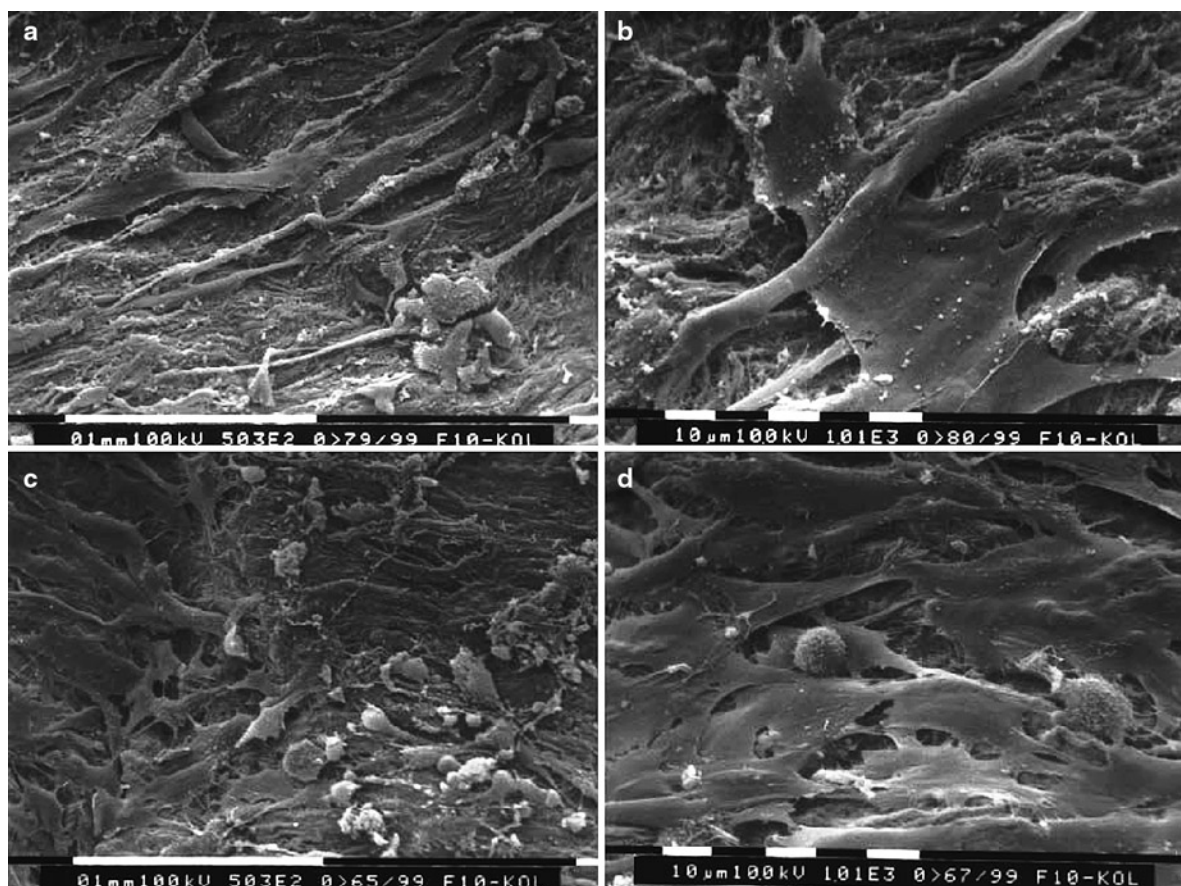


Fig. 1.23 Primary human periodontal ligament fibroblasts (HPLF) and human osteoblast-like cells (SAOS-2) grown on Bio-Gide (BG) barriers; attachment time: 21 days; SEM. (a) HPLF densely grown on the collagen membrane, elongated cells reveal physiologic morphology; original magnification $\times 500$. (b) HPLF cultured on collagen membrane; original mag-

nification $\times 1000$. (c) SAOS-2 cultured on the collagen barrier, numerous adherent cells with physiologic morphology have populated the membrane; original magnification $\times 500$. (d) SAOS-2 densely grown on collagen membrane; original magnification $\times 1000$ (Alpar et al. 2000. Reprinted with permission from of Springer)

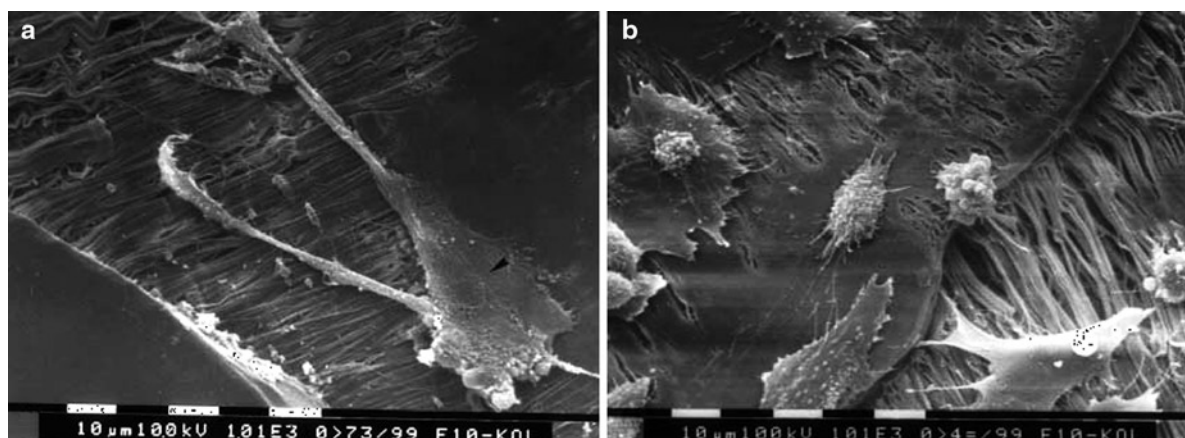


Fig. 1.24 (a, b) Primary human periodontal ligament fibroblasts (HPLF) and human osteoblast-like cells (SAOS-2) grown on nonresorbable polytetrafluoroethylene (ePTFE) barriers; attachment time: 21 days; SEM; original magnification $\times 1000$.

(a) HPLF cultured on the ePTFE membrane, very few flattened cells (arrowhead) are visible. (b) SAOS-2 cultured on the ePTFE barrier; few cells are visible (Alpar et al. 2000. Reprinted with permission from of Springer)

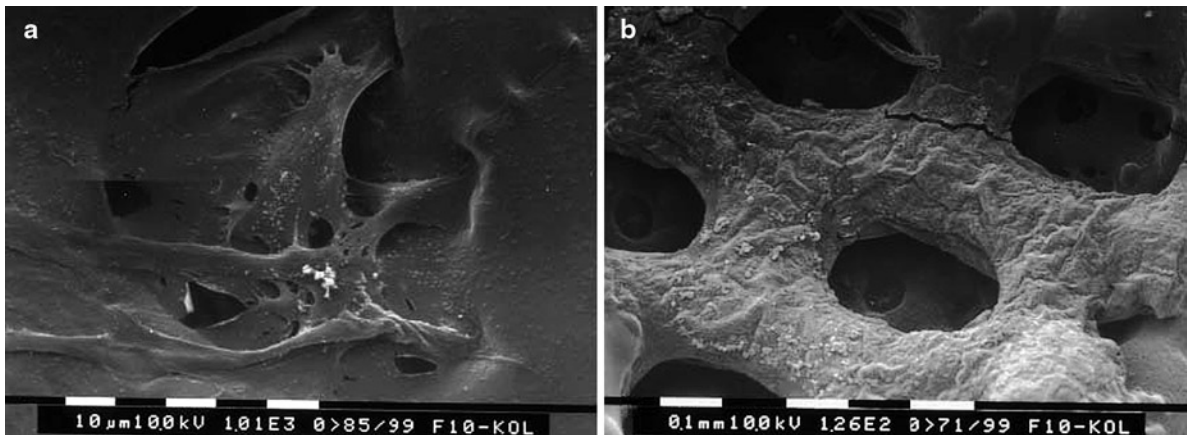


Fig. 1.25 Primary human periodontal ligament fibroblasts (HPLF) and human osteoblast-like cells (SAOS-2) grown on poly(lactic acid) barriers; attachment time: 21 days; SEM. (a) HPLF grown on the poly(lactic acid) barrier. Few elongated cells adhering to the membrane are visible; original magnification

$\times 500$. (b) Poly(lactic acid) membrane 21 days after growth of SAOS-2 cells; even at the lower magnification, which shows a larger area, no adherent cells are visible on the barrier; original magnification $\times 126$ (Alpar et al. 2000. Reprinted with permission from Springer)

Table 1.7 Commercially available collagen membranes

	Company	Collagen origin	Mode of processing
BioBar	Colbar research & dev. Ltd, Ramat-Hasharon, Israel	Bovine (type I)	—
Bicon	Bicon, Boston, MA, USA	Bovine (type I)	—
Bio-Gide	Geistlich Pharma AG, Wolhusen, Switzerland	Porcine (type I and III)	—
BioMend	Zimmer Dental, Carlsbad, CA, USA	Bovine (type I)	Glutaraldehyde cross-linked
BioMend Extend	Zimmer Dental, Carlsbad, CA, USA	Bovine (type I)	Glutaraldehyde cross-linked
Biostite	Coletica, Lyon, France	Calfskin (type I collagen, hydroxyapatite with chondroitin-4-sulfate glycosaminoglycan	DPPA
Opocrin (discontinued)	Vebas, Milano, Italy	Equine (type I (97%) and type III (3%))	DPPA cross-linked
Osseoguard	Biomet, Inc., Warsaw, IN, USA	Bovine (type I)	Non-cross-linked but processed otherwise (corporate secret)
Ossix (discontinued)	(Previously: 3i, Colbar R&D Ltd., Ramat Husharon, Israel)	Bovine (type I)	Enzymatically cross-linked
Ossix Plus	OraPharma, Inc, Warminster, PA, USA	Bovine (type I)	Glycation (Glymatrix) cross-linked
Paroguide (discontinued)	Coletica, Lyon, France	96–98% equine (type I) (97%) and type III (3%) collagen with 2–4% chondroitin-4-sulfate glycosaminoglycan	DPPA cross-linked
Periogen	Collagen Inc. Palo Alto, CA	Bovine dermis (type I and III)	Glutaraldehyde
TissueGuide	KOKEN, Tokyo, Japan	Bovine (type I)	Atelo-collagen
Tutodent	Tutogen Medical GmbH, Neunkirchen am Brand, Germany	Bovine (type I)	—

Source: Behring et al. (2008). Reprinted with permission from Springer.

DPPA diphenylphosphoryl azide

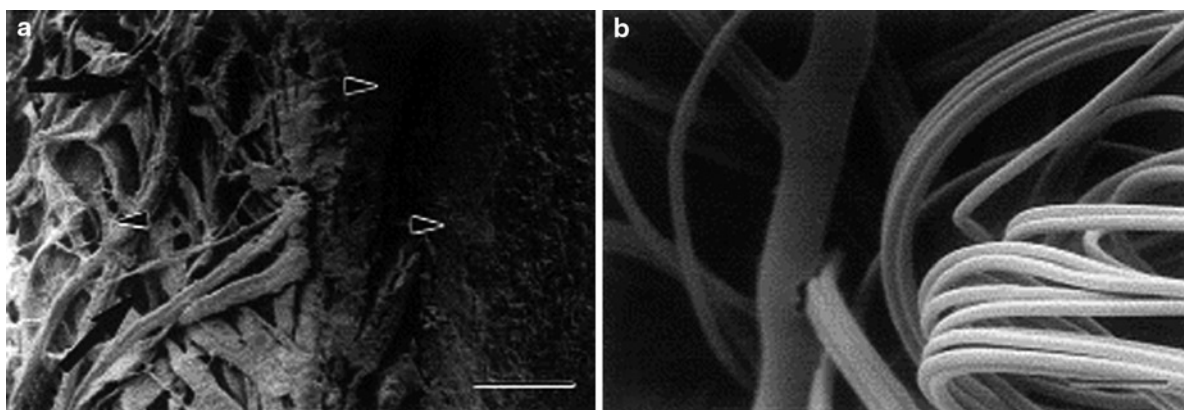


Fig. 1.26 (a) The native material of the Bio-Gide® membrane revealed a bilayer structure with a compact outer layer (arrowheads) and a porous inner layer (arrows) of collagen fiber bundles. SEM, BAR=100 μm. (b) The native material of the

Ethisorb® carrier revealed homogeneously distributed fibers in form of a three-dimensional network (Fig. 1.26b). SEM, BAR=100 μm (Hillmann et al. 2002. Reprinted with permission from Elsevier)

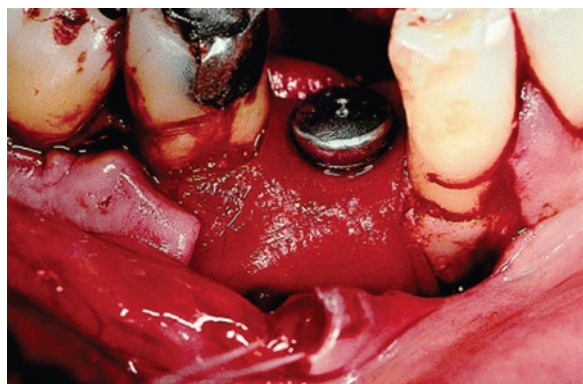


Fig. 1.27 A Bio-Gide® membrane has been adapted around the neck of the implant and is extended onto intact bone beyond the borders of the defect. Bio-Oss® granules are used to support the membrane in the area of the defect. A healing cap will allow the adaptation of the flaps to obtain transmucosal positioning of the implant during the healing phase (Hämmerle and Lang 2001. Reprinted with permission from John Wiley & Sons)

Moses et al. 2005) and intra-bony or furcation defects (Sculean et al. 2003, 2005; Camargo et al. 2000; Tonetti et al. 2004; Hartman et al. 2004; Camelo et al. 1998, 2001; Nevins et al. 2003; Houser et al. 2001) (Fig. 1.28). Similar results were obtained when it was compared with Emdogain in the treatment of periodontal defects (Pietruska 2001).

It was demonstrated that Bio-Gide® membranes were significantly more susceptible to degradation than BioMend membranes. Differences in membrane degradation may be attributed to the use of cross-

linked collagen membranes like BioMend, where collagen is linked by formaldehyde, versus non-cross-linked membranes like Bio-Gide. In general, it has been reported that cross-linked collagen membranes have slower resorption rates than those without cross-linking (Sela et al. 2003).

A type I collagen guided tissue regeneration membrane approved for clinical use is manufactured from collagen derived from bovine deep flexor (Achilles) tendon (*BioMendTM*). This membrane is semiocclusive (effective pore size 0.004 μm), and is paper-white in the dry state with a surface texture similar to leather. In cross-section, the composition of condensed, laminated sheets is visible. The BioMend membrane becomes translucent when hydrated but remains non-slippery and adaptable to the tooth root, which facilitates placement. The BioMend membrane serves as more than an inert barrier. By creating an environment where the wound can heal, the collagen membrane helps stabilize and maintain the blood clot in the defect site. The BioMend membrane absorbs completely in 4–8 weeks into surrounding gingival connective tissue through enzyme (collagenase) degradation. It was found that the membrane may influence the process of bone regeneration in vivo through the effects of their presence on cell migration (Rothamel et al. 2004; Takata et al. 2001b).

Several authors have evaluated this membrane in clinical settings with promising results (Shieh et al. 1997; Wang et al. 1994; Yukna and Yukna 1996).

BioMend ExtendTM (Sulzer Calcitek, Carlsbad, CA, USA) is a type 1 collagen membrane derived from

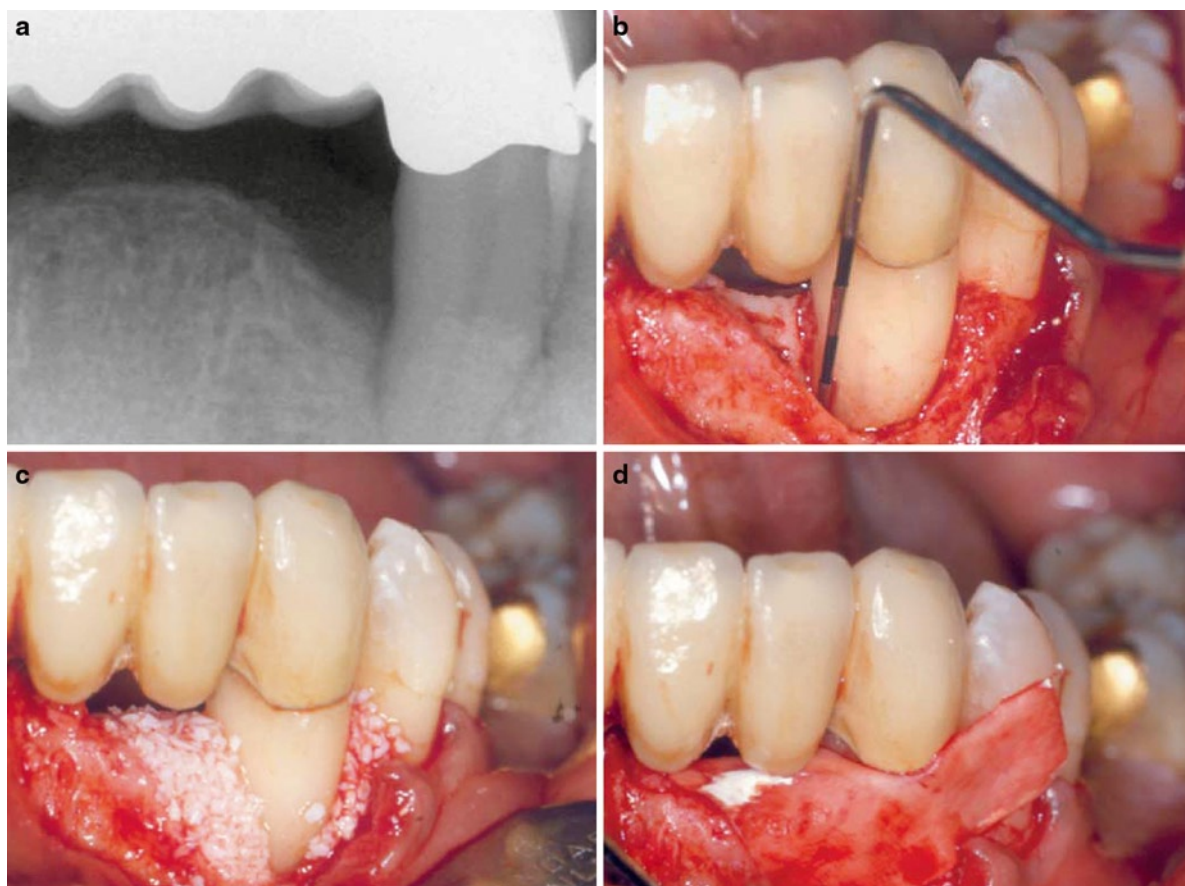


Fig. 1.28 Case treated with Bio-Gide Perio and Bio-Oss. (a) At baseline, there was a radiographic evidence of an intrabony component (IC) ≥ 4 mm. (b) this was confirmed during surgery after debridement of the defect. (c) The defect was filled with

Bio-Oss and (d) was covered with a Bio-Gide Perio membrane (Stavropoulos et al. 2004b. Reprinted with permission from Springer)

bovine tendon and is highly cross-linked to prolong membrane barrier function to maximize the time necessary for tissue regeneration (Rothamel et al. 2004). The BioMend Extend membrane has the same benefits as the BioMend membrane, with the following additional advantages: thicker (excellent handling and mechanical properties, pliable and tear resistant), longer lasting (maintains an effective barrier longer for greater regenerative results) and bioabsorbable (fully absorbed into surrounding tissue within 18 weeks).

Oclastim (*Opocrin* or *Oblastim*) membranes measure 3 $\times$ 3 cm, are made up of pure collagen type I and horse Achilles' tendon extract and are treated with pepsin to eliminate the telopeptide (atelocollagen). The formation of crossed bonds among the parallel fibers was obtained by physical agents and gamma-ray sterilization (Cesari et al. 2006).

OsseoGuard™ is a white, nonfriable membrane matrix engineered from highly purified type I collagen fibers derived from bovine Achilles tendon that is intended for use in periodontal and/or dental surgery procedures as a material for placement in the area of periodontal defects, dental implant, bone defect or ridge reconstruction to aid in wound healing postsurgery. *OsseoGuard™* has a morphology of dense oriented fibers for mechanical strength. Macromolecular permeation studies have shown that the membrane is permeable to macromolecules. Its porosity is such that it effectively retards epithelial downgrowth and prevents gingiva connective cell migration into the wound site. The semipermeability properties of the membrane permit the exchange of essential nutrients for wound healing. The *OsseoGuard* Membrane has a shelf-life of 3 years and is designed for guided bone regeneration

indications such as localized ridge augmentation, peri-implant bone defects around implants, extraction sockets, bone regeneration after root resection and sinus window (www.biomet3i.se/countries/en/directory/misc/OsseoGuard.../english.pdf).

Ossix™ (Implant Innovations, Inc., Palm Beach Gardens, FL, USA) (enzymatic cross-linked bovine type I collagen) offers excellent handling characteristics in guided bone regeneration and predictable clinical outcomes through its patented cross-linking technology. *Ossix* has demonstrated full 6-month barrier function. The tissue-friendly cross-linking reduces inflammation and resists resorption, as it is cross-linked with glucose metabolite, a substance naturally found in the body. This is a significant advantage over synthetic membranes, which may need to be removed, and over other collagen membranes, which may degrade in a matter of days if they become exposed. *Ossix* has several other advantages: it is fully resorbed within 8–10 months, need not be removed when exposed and maintains barrier function for 2–4 months when exposed (Friedmann et al. 2001, 2002; Moses et al. 2005). *Ossix* has the capacity to promote the attachment and proliferation of human periodontal ligament fibroblasts and osteoblasts in vitro (Rothamel et al. 2004) and is biocompatible for cells derived from human periosteum (Warnke et al. 2009). However, the membrane failed to demonstrate any formation of blood vessels in the layers of this membrane during a whole observation period of 24 weeks when angiogenesis pattern was immunohistochemically evaluated after subcutaneous implantation in rats (Schwarz et al. 2006). However, the new collagen barrier *Ossix* was suitable for the technique of guided bone regeneration and, utilized in combination with graft materials, provided a highly predictable healing pattern and augmentation success (Kim et al. 2010; Urban and Wenzel 2010; Moses et al. 2005, 2009; Veis et al. 2006; Friedmann et al. 2002).

Paroguide (Coletica) membranes measuring 3 x 3 cm; 96% of their weight was made up of collagen type I, bovine skin extract, and 4% by chondroitin-4-sulphate, obtained from sheep nasal septum. The membranes were treated with diphenyl-phosphoryl-azide (DPPA), which induced the formation of peptide bonds crossed between free groups of amine and acid collagen without leaving toxic residues on the membrane or changing the triplehelix structure of natural collagen, and sterilized by gamma rays (Cesari et al. 2006). Benque et al. (1997) used with effective results *Paroguide* in

the treatment of deep three-wall intrabony defects in both adult and rapid progressive periodontitis. Parodi et al. (1996) reported histologically verified periodontal ligament, cementum and alveolar bone regeneration, with no signs of inflammation, after insertion of this collagen membrane (*Paroguide®*). When the biocompatibility of several collagen membranes was investigated by Marinucci et al. (2003), it was revealed that the *Paroguide* membrane, which contains chondroitin sulfate, increased cell proliferation more than that by *Opocrin*, *Biomed Extend* and *Bio-Gide*, and a considerably greater amount of organic macromolecules in the extracellular environment, mainly with *Paroguide* and *Opocrin*, was observed. When TGFβ₁ production by human fibroblasts cultured on DPPA- and GA-treated membranes was investigated, it was revealed that the *Paroguide* membrane increased TGFβ₁ secretion more than that by *Opocrin* (Marinucci et al. 2003).

Other natural products tested as guided tissue regeneration devices include (Tatakis et al. 1999):

- *Dura mater*, consisting of an irregular network of collagen fibers, is obtained from cadavers, processed to eliminate antigenic and pyrogenic activity, then lyophilized and sterilized. Use of cadaveric *dura mater* may represent a risk of acquiring Creutzfeldt–Jakob disease (Tatakis et al. 1999).
- *Cargile membranes* are procured from bovine intestines (ox cecum) and processed in a manner similar to chromic gut sutures. The device is difficult to manage, provides limited inhibition of epithelial apical migration, with the material substantially resorbed by 4 weeks postsurgery (Tatakis et al. 1999).
- *Oxidized cellulose mesh*, a hemostatic dressing, can be completely resorbed within 4 weeks of implantation, appears to offer limited, if any, space provision and/or maintenance (Tatakis et al. 1999).
- *Laminar bone*, a 300- to 500-µm thick strip of cortical bone, processed in a manner similar to demineralized freeze-dried bone allografts, has also been used as a guided tissue regeneration device (Tatakis et al. 1999).
- *Acellular dermal matrix (ADM)* (AlloDerm Life Cell Corporation, The Woodlands, TX, USA), material obtained from human skin, has been used as a substitute for palatal connective tissue to increase the width of keratinized tissue around teeth or implants (Wei et al. 2000; Harris 2001; Buduneli

et al. 2003), for the treatment of alveolar ridge deformities (Batista et al. 2001), for root coverage procedures (Aichelmann-Reidy et al. 2001; Novaes et al. 2001; Paolantonio et al. 2002; Woodyard et al. 2004; Barros et al. 2004; De Queiroz Côrtes et al. 2006; Park and Wang 2006; Felipe et al. 2007; Andrade et al. 2008; Pourabbas et al. 2009) (Figs. 1.29–1.31) and for the removal of melanic pigmentation (Novaes et al. 2002a; Pontes et al. 2006; Scarano et al. 2009). A recent meta-analysis on the comparison of ADM to common mucogingival surgical procedures failed to draw any definitive conclusion due to inadequate



Fig. 1.29 Severe decreases in the width of attached gingiva in the upper anterior region. Note the striking displacement of free gingiva with lip retraction in the upper left central incisor (arrow) (Buduneli et al. 2003. Reprinted with permission from John Wiley & Sons)



Fig. 1.30 Allograft secured to the firm periosteal bed by 5-0 chromic gut sutures, with the basement membrane complex side facing up toward the vestibule (Buduneli et al. 2003. Reprinted with permission from John Wiley & Sons)

available data and need for further randomized clinical studies emphasized. However, there were no statistically significant differences between groups for any of the outcomes measured (recession coverage, keratinized tissue formation, probing depths and clinical attachment levels). Considering the heterogeneity values found among the studies, certain trends could be found: (a) three out of four studies favored the ADM group for recession coverage; (b) a connective tissue graft tended to increase keratinized tissue compared to ADM (0.52-mm difference; $P=0.11$); (c) there were trends of increased clinical attachment gains comparing ADM to coronally advanced flap procedures (0.56-mm difference; $P=0.16$) (Gapski et al. 2005). In a dog model, ADM acted as a barrier in guided bone regeneration, with clinical, radiographic and histomorphometric results similar to those obtained with the bioabsorbable membrane (Borges et al. 2009). The ADM also demonstrated histomorphometric results similar to the bioabsorbable membrane and resulted in a greater increase in the thickness of the keratinized tissue in the treatment of mandibular class II furcation lesions (Andrade et al. 2007). Another indication of the ADM membrane was guided bone regeneration in edentulous ridges and in association with immediate implants (Novaes and Souza 2001; Novaes et al. 2002b; Luczyszyn et al. 2005; Fowler et al. 2000a, b; Griffin et al. 2004).

- One of the oldest biomaterials used for scaffolds is the fetal membrane. In particular, *amniotic membrane (AM)* has gained importance because of its ability to reduce scarring and inflammation, enhance wound healing, and serve as a scaffold for cell proliferation and differentiation as a result of its antimicrobial properties. In addition, the AM is a biomaterial that can be easily obtained, processed and transported (Niknejad et al. 2008). AM was used as a barrier membrane between the gingival epithelium and hard tissue to promote the periodontal ligament cells to form progenitor cells that can regenerate new tissues in the treatment of human periodontal grade II buccal furcation defects (Kothiwale et al. 2009). AM was also effective in helping cicatrization and wound healing after dental implant surgery. AM supports the growth of epithelium thus facilitating migration and reinforcing adhesion. It decreases pain (Velez et al. 2010). The amniotic

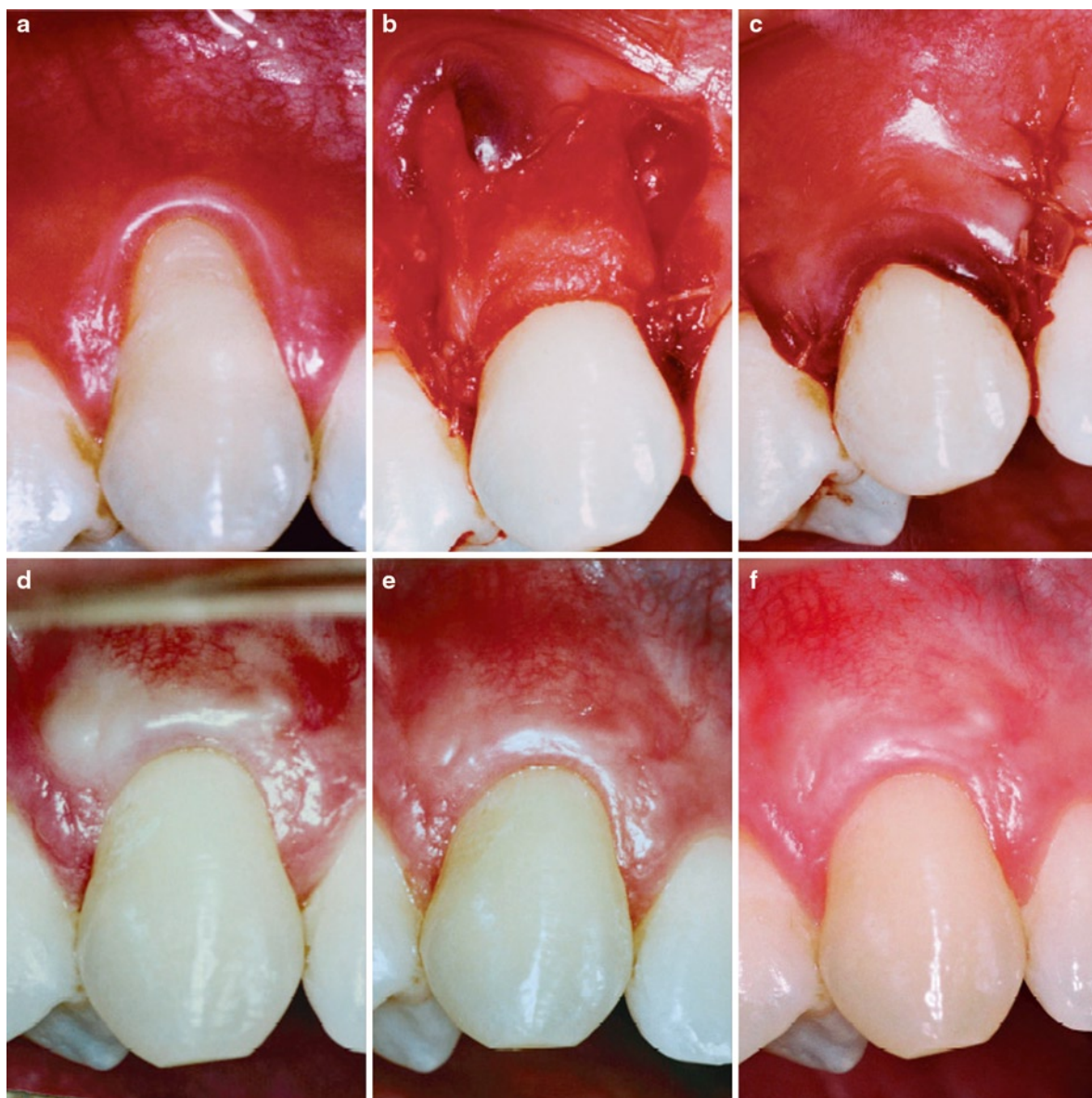


Fig. 1.31 Site treated with acellular dermal matrix (ADM) allograft. (a) Initial clinical view of the gingival recession. (b) ADM sutured in place. (c) Suture of the coronally positioned flap, completely cover-

ing the graft. (d) Six months after the surgery. (e) One year after surgery. (f) Two years after the surgery (de Queiroz Côrtes et al. 2006. Reprinted with permission from John Wiley & Sons)

membrane is a thin structure and has technical limitations with regards to suturing. The stiff character of AM allows it to be adapted easily to the defect site over the graft material in the surgical site without shriveling and suturing (Niknejad et al. 2008). Also AM demonstrates increased stiffness that enhances the membrane strength necessary to resist strength induced during the growth of the tissue (Kothiwale et al. 2009).

1.7.3.2 Synthetic Products

Synthetic resorbable materials are usually organic aliphatic thermoplastic polymers. The materials most commonly used are poly- α -hydroxy acids, which include polylactic polyglycolic acid and their copolymers (Figs. 1.32 and 1.33). One of the advantages of polyhydroxy acid is hydrolysis to final products water and carbon dioxide. Degradation time can vary,

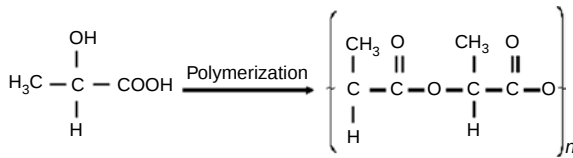


Fig. 1.32 Poly(lactic acid)

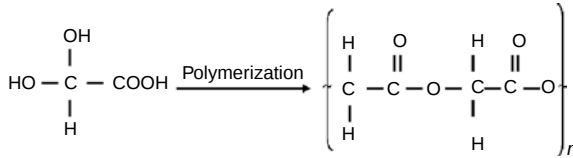


Fig. 1.33 Poly(glycolic acid)

lengthened through the addition of lactides or glycols (Aurer and Jorgic-Srdjak 2005).

A double-layered absorbable membrane, *Guidor*[®] (Guidor Bioabsorbable Matrix Barrier, Guidor, Huddinge, Sweden), made of *polylactic acid* and a *citric acid ester acetyl tributylcitrate* was the first to appear on the market. The external layer of the membrane designed to allow integration of the overlying gingival flap has rectangular perforations (400–500/cm²). Between the internal and external layers are internal spacers creating space for tissue ingrowth. The internal layer has smaller circular perforations (4,000–5,000/cm²) and outer spacers for maintaining the space between the membrane and the root surface. Due to this bilayered matrix structure, the membrane facilitates connective tissue integration, avoiding epithelial downgrowth at the gingiva-faced surface (Christgau et al. 1997). A complete resorption of membrane 6–12 months after implantation and maintenance of function for at least 6 weeks were reported. The degradation process includes foreign-body reaction characterized by macrophages and multinuclear cells (Aurer and Jorgic-Srdjak 2005). Several clinical studies have evaluated the Guidor membrane in guided tissue regeneration therapy (Rüdiger et al. 2003; Wang et al. 2002; Mehlbauer et al. 2000; Grimm et al. 2000; Ratka-Kruger et al. 2000; Pontoriero et al. 1999; Vernino et al. 1998, 1999; Parashis et al. 1998; Nickles et al. 2010) (Fig. 1.34).

Other synthetic bioabsorbable barriers that are currently in the market include *Resolute*[™]. This regenerative material manufactured by Gore consists of an occlusive film made of polylactic and polyglycolic

acids with a ratio of 85/15. The fibers of pure glycolic acid are attached to the occlusive film in a randomized fashion to accomplish integration with the flap tissue. *Resolut XT*[™] is an improved version of *Resolute*[™]. It consists of polylactic and polyglycolic acid with the addition of trimethylene carbonate, another synthetic polymer which delays the process of initial resorption (AlGhamdi and Ciancio 2009). *Resolut Adapt*[®] LT Regenerative Membrane (Gore-Tex; W.L. Gore & Associates, Inc., Flagstaff, AZ, USA) consists of a three-layer structure of two random fiber matrices on either side of a cell-occlusive film. The membrane is composed of the synthetic polymers: polyglycolic acid, polylactic acid and trimethylene carbonate. The occlusive membrane part serves the cell-exclusion function, the porous structure serves the tissue integration function while the stiffness of the device serves the space maintenance function. It is supplied with a polycaprolate-coated polyglycolic acid (*Resolut*) suture, used to secure the device to the tooth. *Resolut LT* Regenerative Membrane remains substantially intact for 8–10 weeks and is substantially resorbed in 6–7 months (Tatakis et al. 1999). The polymer components of this barrier hydrolyze, are safely resorbed in body tissue and have been found to be inert, nonantigenic, nonpyrogenic and elicit only a mild tissue reaction during bioabsorption (Caffesse et al. 1997). Histological and histometrical evaluation of this bioresorbable barrier in beagle dogs and in monkeys proved the benefits of this barrier in facilitating periodontal regeneration (Caffesse et al. 1994; Warrer et al. 1994; Hürzeler et al. 1997; Quinones et al. 1994). It has been showed that the use of bioabsorbable membranes composed of a polylactide/polyglycolide copolymer (*Resolut*) resulted in clinically and statistically significant improvements in recessions, probing depths and attachment levels, in interproximal intrabony defects and class II furcation defects (Fig. 1.35) (Tonetti et al. 1998; Sanz et al. 1997; Vincenzi et al. 1998; Caffesse et al. 1997; Danesh-Meyer et al. 2002; Windisch et al. 1999, 1998; Sculean et al. 1999b, 1999c; Vincenzi et al. 1998; Stavropoulos et al. 2004b). Favorable results were also reported in bone augmentation (Donos et al. 2002; Stavropoulos et al. 2004c; Pretorius et al. 2005).

OSSEOQUEST[™] is another synthetic barrier by Gore. It has the same consistency as *RESOLUT XT*[™] but it takes a longer time to disintegrate (16–24 weeks)

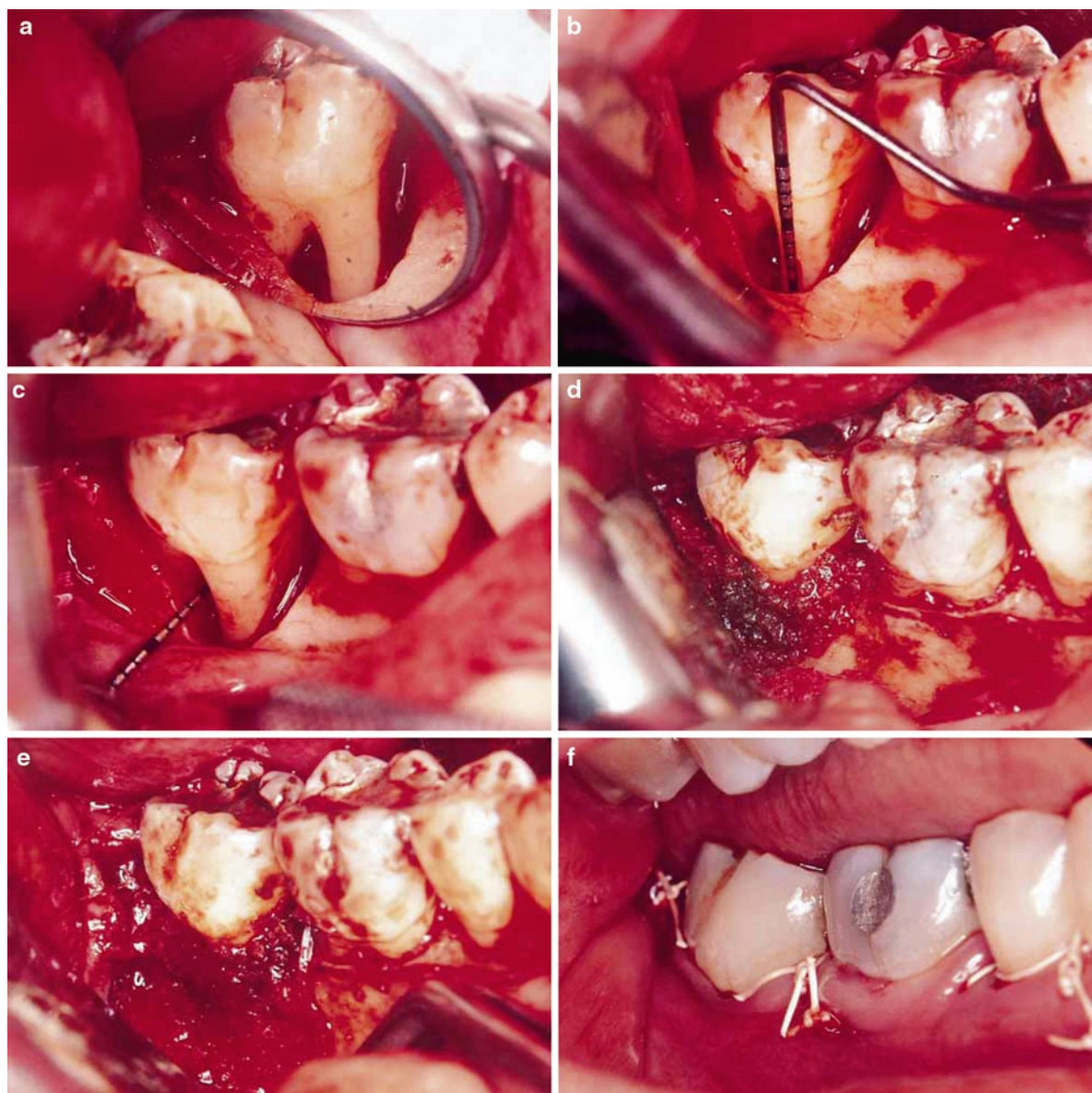


Fig. 1.34 Management of combination class II furcation, intrabony (F and M), and dehiscence osseous defects with combined regenerative therapy employing demineralized freeze-dried bone allografts and a bioabsorbable barrier membrane (Guidor™). (a) Facial (mirror) view of tooth 47 after flap reflection and debridement depicting mesial and facial osseous defects. (b) Probe (15 mm) depicting 5 mm intrabony defect and 7 mm dehiscence to the cementoenamel junction on mesial root. (c) 5-mm horizontal

furcal defect (class II). (d) Demineralized freeze-dried bone allograft in position. (e) Guidor™ in position. (f) Flap closure and membrane coverage. (g) Six-week postoperative appearance. (h) Eight-month postoperative with 2-mm probing attachment level in furcation. (i) Preoperative radiograph. (j) Eight-month postoperative radiograph depicting bone fill correlating with clinical improvement in probing attachment levels (McClain and Schallhorn 2000. Reprinted with permission from John Wiley & Sons)



Fig. 1.34 (continued)

(AlGhamdi and Ciancio 2009; von Arx et al. 2005). The GORE OSSEOQUEST Regenerative Membrane has a unique hexametric pattern and open surface structure that provides an environment for optimal tissue integration, which may increase flap integrity and lessen the chance of early exposure. The open microstructure and hexametric pattern combine to give the surgeon an easy-to-place membrane (<http://www.dentalcompare.com/details/4556/Gore-Ossequest.html>). The glycolide–lactide–trimethylene carbonate Osseoquest membrane is used in guided bone regeneration, ridge augmentation and implants (Roos-Jansåker et al. 2007a, b; Sommerlad et al. 2007; Veis et al. 2004).

The fiber of *polyglactin 910*, a copolymer of glycolide and L-lactide (90/10 molar ratio), was used to prepare a tightly woven mesh (*Vicryl Periodontal Mesh™*; Vicryl-Netz Parodontalzuschnitt, Ethicon, Norderstedt, Germany). To facilitate anchoring of the device onto the tooth, it is manufactured with polyglactin 910 sutures placed through its coronal

margin(s). These membranes have no special structure for connective tissue integration at the gingiva-faced surface and degrade in 4–6 weeks (Tatakis et al. 1999). Clinical evaluation of the polyglactin 910 device suggests that it is effective in a variety of defects: gingival recessions (Gupta et al. 2006), furcations (Mehlbauer et al. 2000) and intrabony defects (Christgau et al. 1995, 1997b, 1998; Eickholz et al. 1997; Stavropoulos and Karring et al. 2004; Stavropoulos et al. 2004).

The Atrisorb barrier® (DL-lactide polymer, Atrix Laboratories Inc., Fort Collins, CO) is the only approved guided tissue regeneration device to be manufactured chairside. The poly(DL-lactide) polymer is supplied in a flowable formulation, dissolved in *N*-methyl-2-pyrrolidone. The polymer accounts for 37% of the formulation and the solvent for 63%, by weight. An irregularly shaped barrier is formed after exposure of the polymer to 0.9% saline solution for 4–6 min in a special cassette. The desired shape and



Fig. 1.35 Management of class II mandibular facial furcation defects with combined therapy employing a bioabsorbable barrier membrane (Resolut™). (a) 4-mm horizontal defect, tooth number 46. (b) Graft and membrane in position. (c) Closure. (d) One-year postoperative evaluation depicting complete furcation fill clinically.

(e) Preoperative radiograph. (f) One-year postoperative radiograph suggesting bone fill in the furcation that correlates with the gain in clinical attachment level. (g) Six-year postoperative radiograph depicting stability (McClain and Schallhorn 2000. Reprinted with permission from John Wiley & Sons)

size is cut and trimmed. The resulting 600–750 μm thick device has modest adhesion properties and is placed into the defect using gentle pressure to position it without suturing (Tatakis et al. 1999). *Atrisorb®-D FreeFlow™* GTR Barrier is the only barrier that contains an antibiotic (doxycycline 4%), provides controlled release of doxycycline for a period of 7 days and is proven to prevent bacterial colonization of the barrier (www.atrisorb.com).

Animal studies have shown that Atrisorb membranes have good tissue response, biocompatibility, and safety (Bogle et al. 1997; Coonts et al. 1998; Polimeni et al. 2008). Histomorphometric analyses of treated sites compared to sham controls showed that the polymer barrier is effective in promoting bone and cementum regeneration in periodontal defects in dogs (Coonts et al. 1998). Regeneration (new bone, cementum and periodontal ligament) of 71% of the natural occurring buccal class II furcation defects in experimental sites and only 14% in control sites in a dog model demonstrated a response that highly favored use of the barrier (Bogle et al. 1997). Periodontal healing after use of the Atrisorb barrier material (polylactic acid) for guided tissue regeneration was studied in surgically induced periodontal defects or caused by naturally occurring periodontitis in beagle dogs. The barriers fragmented and became displaced in 2–5 weeks after application. At re-entry after 4 months, it was revealed that new bone covered 60–100% of the formerly exposed furcations and root surfaces. Sites obtained for histologic evaluation 9–12 months after the baseline surgery showed new connective tissue attachment, cementum and alveolar bone. Histomorphometric analyses quantitated these tissue changes, and new connective tissue attachment covered 72% of surgically exposed root surfaces and 77% of periodontitis-exposed root surfaces (Polson et al. 1995b).

Several clinical studies have evaluated the clinical efficacy of Atrisorb membrane in the guided tissue regeneration treatment of intrabony and furcation defects or for dehiscence and fenestration defects on implants (Sakallioğlu et al. 2007; Bremm et al. 2004; Polson et al. 1995a, c; Garrett et al. 1997; Rosen et al. 1998; Rosen and Reynolds 1999, 2001; Hou et al. 2004; Nygaard-Østby et al. 2008). Atrisorbs has been reported to be able to achieve results equivalent to those of ePTFE (Garrett et al. 1997).

Epi-Guide® is membrane made of *polylactic acid polymers*, has *three layers* designed to stop and keep away epithelial cells and fibroblasts:

- Layer 1: Defect Interface: limited porosity supports a vigorous uptake of fluid blood, facilitates adherence to the tissue surface and prevents fibroblasts from reaching the defect.
- Layer 2: Inner Surface: the inner labyrinth catches fibroblasts and forces them into random pathways while inner chambers enable collateral circulation and unrestricted flow of interstitial fluid through the membrane.
- Layer 3: Gingival Interface: Numerous voids and interconnected pathways decelerate fibroblast infiltration, promoting cell attachment

It maintains its structure for 20 weeks, and is fully resorbed in 6–12 months (Aurer and Jorgic-Srdjak 2005; Bilir et al. 2007). In animal studies, created periodontal defects in baboons treated with GTR showed after 6 weeks more gingival recession at the Gore-Tex sites than at the Epi-Guide sites. At 6 weeks, the Epi-Guide material was present histologically in a partially resorbed state. There was a mild inflammatory reaction in the surrounding connective tissues (Vernino et al. 1995). In cell-culture studies, Epi-Guide has been proven to show good biocompatibility with human osteoblast-like cells (Bilir et al. 2007) (Fig. 1.36), osteoblastic cells (Takata et al. 2001b) and periodontal ligament cells (Takata et al. 2001a). In clinical studies, over a 12-month period, the composite reduction in the vertical component of the osseous defects was greater in the sites treated with Epi-Guide as compared to those treated with Guidor (Vernino et al. 1999).

Experimental Mempol® membrane manufactured from polydioxanone (PDS), a dioxanone polymer, is bilayered. The first layer is completely unpermeable, covered with PDS loops 200 μm long on the gingival side, intended for integration with connective tissue. On one side of this membrane is a layer of slings. This side is to be placed toward the mucoperiosteal flap. The slings shall facilitate tissue integration and thereby prevent flap dehiscence and barrier exposure. The barrier is adapted to the particular tooth by a Polyglactin 910 suture (Vicryl™, Ethicon GmbH & Co. KG, Norderstedt, Germany) (Aurer and Jorgic-Srdjak 2005). No significant differences were observed when the membrane was compared with a biodegradable polylactide

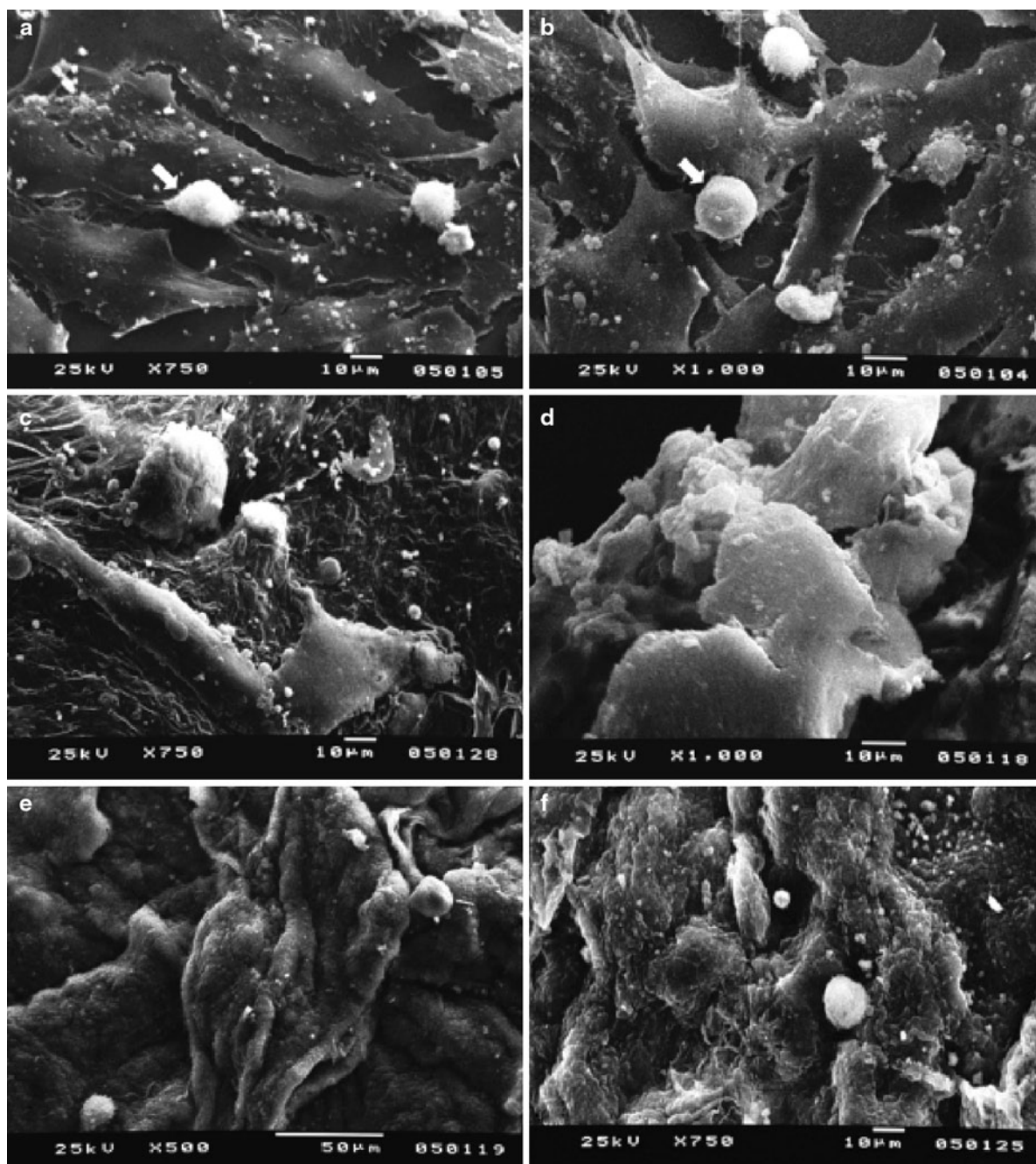


Fig. 1.36 Scanning electronmicrograph (SEM) images of the CRL11372 cells cultured on culture dishes (CD; positive controls), Polylactic acid (Epi-Guide®; EG) membrane and collagen membrane (Bio-Collagen®, BC) membrane groups at the end of 24 and 72 h. (a) SEM of CRL11372 osteoblast cells showing large cytoplasm that are connected to each other via extensions (CD group, 24 h, original magnification $\times 750$). (b) Cells after 72 h in the CD group (original magnification $\times 1,000$). (c) SEM

showing a polygonal shape having mitotic figures (EG group, 24 h, original magnification $\times 750$). (d) Cells after 72 h on the EG membrane (original magnification $\times 1,000$). (e) Cells after 24 h on the BC membrane group (BC group, 24 h, original magnification $\times 500$). (f) Cells after 72 h on the BC membrane group (original magnification $\times 750$) (Bilir et al. 2007. Reprinted with permission from John Wiley & Sons)

acetyltributyl citrate barrier (Guidor Matrix Barrier, Guidor AB, Huddinge, Sweden) in three- and two-wall intrabony defects (Dörfer et al. 2000; Christgau et al. 2003).

Besides the already mentioned polyester membranes, use of *polyurethane* for membrane production has been described (Tatakis et al. 1999; Aurer and Jorgic-Srdjak 2005). Polyurethanes are organic polymers containing urethane group —NH—CO—O— , materials with diverse properties. Polyether urethanes are degraded through enzymatic and oxidative degradation. The membrane seems to be present in the tissue for at least 8 weeks after implantation (Leghissa and Botticelli 1996; Warrer et al. 1992; Aurer and Jorgic-Srdjak 2005).

It has been observed that mineralized collagen exhibits different physical and mechanical properties with those of pure collagen. Moreover, mineralized collagen is considered to provide better biocompatibility toward animal and human tissues than pure collagen. As a hard tissue implant material, mineralized collagen also has a conductivity effect that enhances hard tissue (e.g., bone) growth. Further, changing the content of calcium phosphate minerals could control the bioresorption rate of mineralized collagen membrane. In recent years, the biomimetic mineralized collagen material, called nano-apatite/collagen composite, prepared by different methods attracted researchers' attention, and a modified polyglactin 910 membrane was presented by Liao et al. (2005, 2007). *The three-layered graded membrane* has one face of 8% nano-carbonated hydroxyapatite/collagen/poly(lactic-co-glycolic acid) (nCHAC/PLGA) porous membrane, the opposite face of pure lactic-co-glycolic acid non-porous membrane, the middle layer of 4% nCHAC/PLGA as the transition through layer-by-layer casting method (Fig. 1.37). As we know, higher carbonated minerals are in accord with higher osteoconductivity and earlier bioresorption. Liao et al. (2007) selected nCHAC as the bioactive component, PLGA as the barrier to prepare one novel three-layered membrane comprising a pliable not brittle, substantially cell-impermeable polymeric layer, a first cell-permeable outer layer superimposed on a first surface of the outer layer, and a second cell-permeable outer layer superimposed on a second surface of the inner layer, opposite the first face. The nano-carbonated hydroxyapatite/collagen material facilitated reliable bone regeneration by inducing undifferentiated cells in the graft recipient site to become osteoblasts

and form new bone (i.e., stimulating cellular transformation). The composite also supplied a ready source of calcium for rapid mineralization (Fig. 1.38). The barrier material is quick and easy to use during surgery and is completely biodegradable, eliminating the need for a second surgery to remove the barrier material (Liao et al. 2005, 2007).

1.7.4 New Trends in Guided Tissue Regeneration Barriers Development

Products used for GTR should maintain biocompatibility, but develop better efficacy, possibly using new techniques and technologies that have been developed and applied in neighboring medical branches. Application of specific adhesion molecules should lead to tissue selection on the membrane surface. Addition of antimicrobial substances might minimize the influence of microbial contamination on regenerative outcome; growth factor incorporation should stimulate regenerative biologic potential of bone and cementum. Combination of these molecules might lead to significant changes in the outcome of GTR procedures (Aurer and Jorgic-Srdjak 2005).

1.7.4.1 Addition of Antimicrobial Substances

Controlling the bacterial colonization in the early healing phase and reducing the spread of infections may increase the predictability of results. The use of systemic antibiotics has therefore been propagated by many researchers investigating regenerative procedures for the treatment of intraosseous defects (Van Winkelhoff et al. 1996; Cortellini and Tonetti 2000; Kornman and Robertson 2000). Compared with systemically delivered antibiotics, locally controlled-release delivery devices applied professionally may exhibit several benefits: independence of patient compliance, enhanced or improved pharmacokinetic response, greater access and the ability to position the drug adjacent to the disease and to deliver a lower total dosage of the drug to the patient but giving a more controlled concentration at the diseased site. Tetracycline or flurbiprofen loaded membrane was

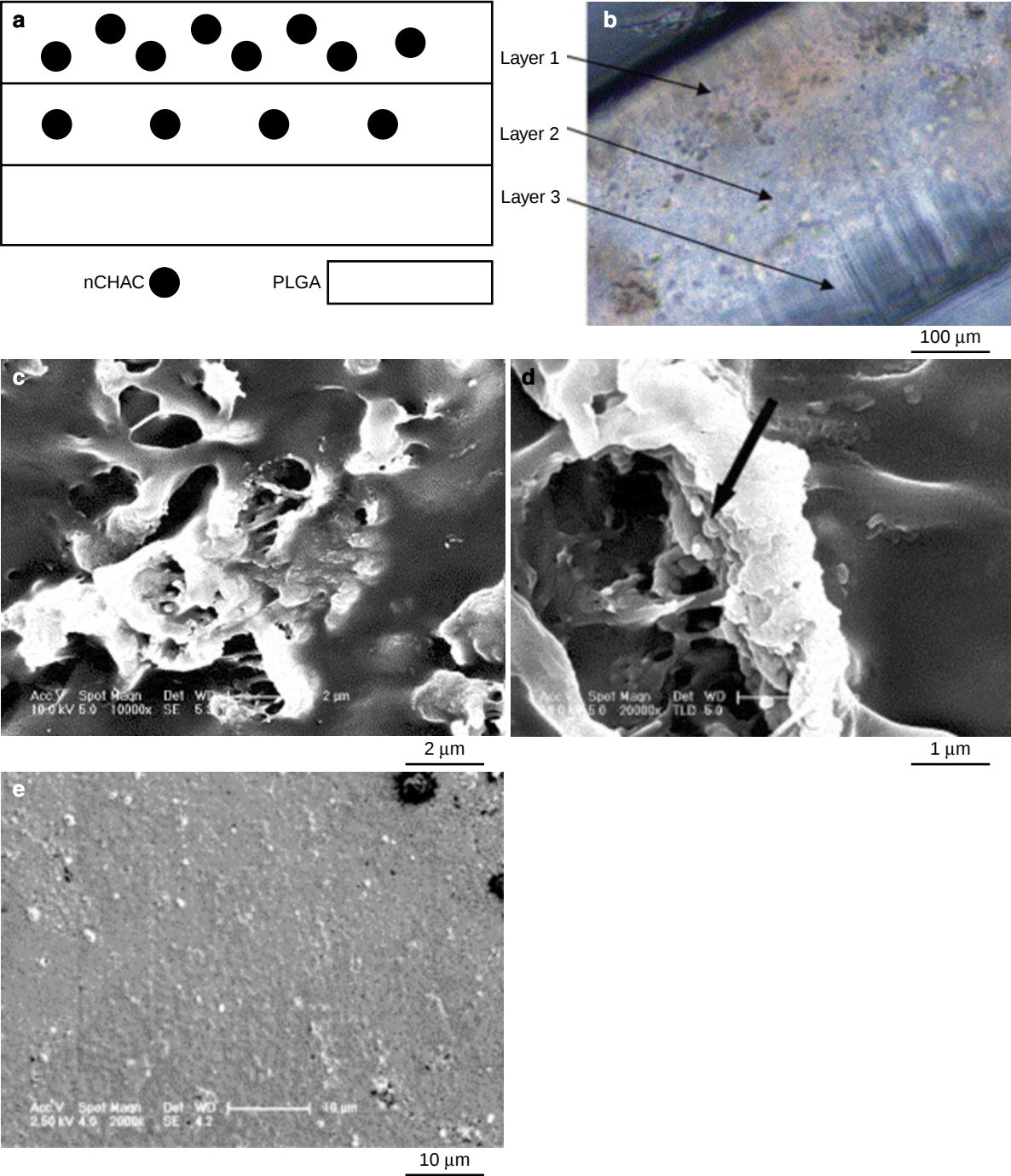


Fig. 1.37 (a) Schematic representation of three-layered membrane (TLM). (b) The OM result of the section of the nCHAC/PLGA TLM. (c, d) The SEM results of Layer 3. The *black arrow*

refers to the nCHAC particles covered by polymer. (e) The SEM result of the underside of Layer 1 (Liao et al. 2005. Reprinted with permission from Elsevier)

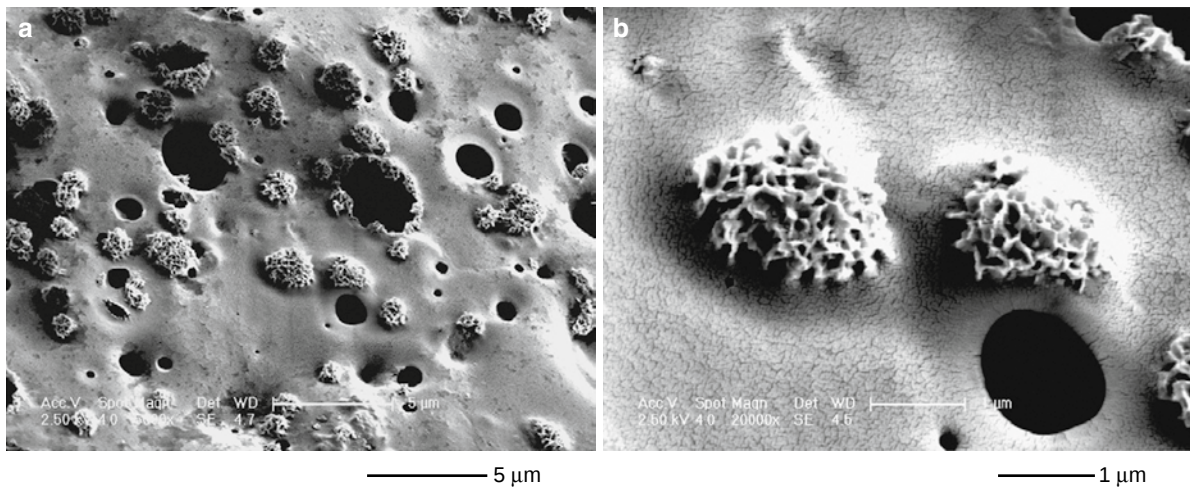


Fig. 1.38 The new nano-apatite formation on the porous surface of the nCHAC/PLGA three FGM layer membrane at 2 weeks observed by SEM, (b) enlarged central part of (a) (Liao et al. 2007. Reprinted with permission from Elsevier)

markedly effective for osteoid tissue and new bone formation in the bony defect prepared in rat calvaria to compare with that by unloaded membrane (Chung et al. 1997). It was demonstrated that incorporation of amoxicillin or tetracycline into various GTR membranes may enhance the attachment of periodontal ligament cells in the presence of the oral pathogens *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* (previously *Actinobacillus actinomycetemcomitans*) (Hung et al. 2005). Cheng et al. (2009) showed that penetration of *S. mutans* and *A. actinomycetemcomitans* through amoxicillin- or tetracycline-loaded ePTFE membrane, glycolide fiber membrane, and collagen membrane was delayed and/or reduced. Thus, incorporation of an antibiotic into the membrane may be of value when controlling membrane-associated infection during GTR therapy.

The tetracycline-coated ePTFE membranes were evaluated in a clinical study by Sipos et al. (2005). However, except for more postoperative discomfort at the MEMP sites, no significant differences were found between enamel matrix protein (Emdogain Gel®) sites and defects that received randomly, as an adjunct to Emdogain treatment, a tetracycline-coated ePTFE membrane (MEMP). The tetracycline-coated ePTFE membranes were prepared in the following way: type GTA 1 and GTA 2 ePTFE (Gore-Tex®, W. L. Gore & Associates Inc., Flagstaff, AZ, USA) barrier membranes were submerged for 1 min in 5% solution of tridodecylmethylammonium chloride (TDMAC) in

absolute alcohol. After drying at room temperature, the TDMAC-coated membranes were immersed for 1 min into a freshly prepared 3% tetracycline solution (250 mg dissolved in 7.5 mL distilled water, pH adjusted from 1.7 to 9.5 using sodium hydrochloride) and dried at room temperature. Tetracycline-coated ePTFE membranes were stored in separate sterile containers in the dark at 10°C (Zarkesh et al. 1999).

However, tetracycline-loaded poly(L-lactide) membranes markedly increased new bone formation in rat calvarial defects and induced bony reunion after 2 weeks of implantation. Biodegradable barrier membranes composed of porous poly(L-lactide) (PLLA) films cast on poly(glycolide) (PGA) meshes were fabricated using an in-air drying phase inversion technique. PLLA was dissolved in methylene chloride–ethylacetate mixtures, cast on knitted PGA mesh, and then air-dried. Tetracycline was incorporated into the membranes by adding it to PLLA solutions (Park et al. 2000).

The Atrisorb®-D FreeFlow™ GTR Barrier is the only barrier that contains an antibiotic – doxycycline 4% – provides controlled release of doxycycline for a period of 7 days and proven to prevent bacterial colonization of the barrier (www.atrisorb.com). It had FDA approval in 2001, and is the first and only GTR barrier that has doxycycline to add antimicrobial and anti-metalloproteinase activity to the barrier function. It is supplied as a two-syringe system: one syringe contains 715 mg ATRIDOX polymer and the other

contains 35 mg doxycycline. The polymer acts as a barrier and as a carrier for the antibiotic, which allows its slow release for up to 10 days (AlGhamdi and Ciano 2009).

The regenerative effect of a 25% *doxycycline-loaded biodegradable GTR membrane* (Doxy-M) was evaluated by Chang and Yamada (2000) in dogs. The Doxy-M-treated defects showed more pronounced new bone formation and less crestal bone resorption than using the simple biodegradable membrane defects. However, the use of a 4% doxycycline hyclate-loaded poly(DL-lactide) polylactic acid barrier did not enhance the treatment outcome compared to a similar non-antibiotic-loaded barrier or bone graft alone (Lyons et al. 2008).

To release drug in an efficient quantity for a long time, (Leprêtre et al. 2007) proposed to modify polyvinylidene difluoride membranes by fixing 14%-wt cyclodextrin polymer with the use of citric acid as cross-linking agent. Complexation of chlorhexidine by β , γ , hydroxypropylated β cyclodextrin was studied. When kinetic release of the chlorhexidine was performed, it was revealed that raw membranes released all chlorhexidine stocked in few hours, whereas grafted membranes released more than tenfold this quantity during 60–80 days. Similar results were reported also by Tabary et al. (2007). Chen et al. (2003) demonstrated that incorporation of chlorhexidine into GTR membranes is beneficial in reducing bacterial effects on cellular attachment.

The regenerative potential of a *metronidazole-loaded biodegradable* (polylactide/glycolide) (PLGA) GTR membrane was evaluated in treatment of created osseous defects in dogs. The histometric analysis at 60 days revealed statistically significantly greater new cementum height, new bone height and new gingival connective tissue height in the experimental groups than the controls (PLGA membranes with or without metronidazole), and control defects showed longer apical extension of junctional epithelium than the experimental defects (Kurtiş et al. 2002). Using a metronidazole-loaded collagen membrane increased the comfort of patients following GTR surgery; however, there was no statistically significant difference in periodontal regeneration when using metronidazole-loaded cross-linked human type I collagen membranes during GTR therapy (Dowell et al. 1995). Recently, different membrane designs loaded with metronidazole were proposed. Metronidazole and amoxicillin were loaded in poly(DL-lactide-co-glycolide) (PDLGA) and poly(DL-lactic acid) (PDLLA) films. The drug-release

study showed that during the first 16 days, the released quantities of drugs were higher than the minimum inhibitory concentration (MIC) needed against various microbes causing periodontal diseases (Ahuja et al. 2006). Shifrovitch et al. (2009) developed and studied metronidazole-loaded 50/50 poly(DL-lactide-co-glycolide) (PDLGA), 75/25 PDLGA and poly(DL-lactic acid) (PDLLA) films. These films were designed to be inserted into the periodontal pocket and treat infections with controlled-release metronidazole for >1 month. The developed systems demonstrated good biocompatibility and the ability to inhibit *Bacteroides fragilis* growth. When exposed to human gingival fibroblasts in cell culture conditions, these films maintained their normal fibroblastic features (Shifrovitch et al. 2009). Bottino et al. (2011) proposed a novel functionally graded membrane (FGM), designed and fabricated via sequential multilayer electrospinning (Fig. 1.39).

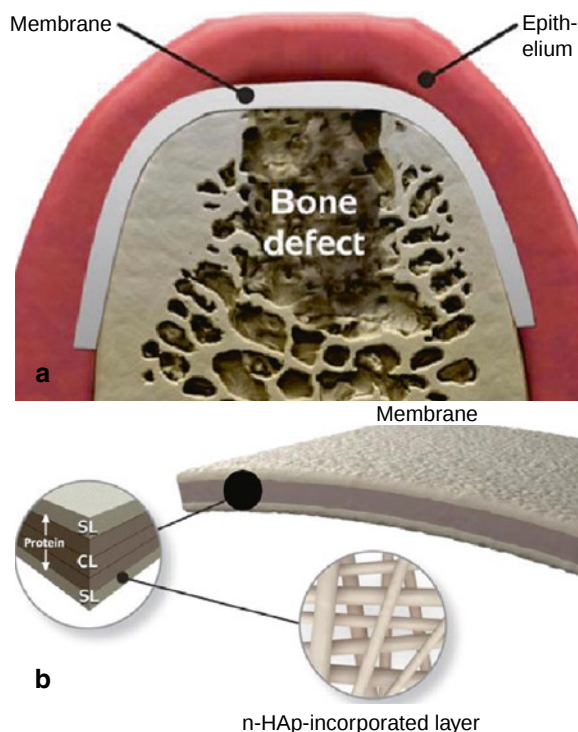


Fig. 1.39 Schematic illustration of the spatially designed and functionally graded periodontal membrane. (a) Membrane placed in a guided bone regeneration scenario. (b) Details of the core layer (CL) and the functional surface layers (SLs) interfacing bone (n-HAp) and epithelial (MET) tissues. Note the chemical composition step-wise grading from the CL to SLs, i.e., polymer content decreased and protein content increased (Bottino et al. 2011. Reprinted with permission from Elsevier)

The FGM consists of a core layer and two functional surface layers interfacing with bone (nano-hydroxyapatite, n-Hap) and epithelial (metronidazole, MET) tissues. The core layer comprises a neat poly(DL-lactide-co-ε-caprolactone) (PLCL) layer surrounded by two composite layers composed of a protein/polymer ternary blend (PLCL:PLA:GEL). Incorporation of n-HAp to enhance osteoconductive behavior and metronidazole to combat periodontal pathogens led to a novel FGM that holds promise at solving the drawbacks of currently available membranes.

1.7.4.2 Addition of Growth and Differentiation Factors

Addition of growth and differentiation factors that applied locally in an adequate vehiculum seem to act on differentiation and cell migration to the wound space.

A bilamellar membrane constituted of an asymmetric poly(L-lactide) acid membrane and an alginate film was presented by Milella et al. (2001). The surfaces of the two membrane sides were different, as expected: the alginate side, to be used in contact with the bone defect, was rougher than poly(L-lactide) acid ones so as to enhance the osteogenesis. When in contact with complete culture medium, the PLLA–alginate membrane retained its mechanical and structural properties for more than 100 days. Then the degradation processes occurred, but the membrane continued to be stable and manageable for 6 months. *Growth factors such as TGF-β* can be incorporated into alginate membranes functioning as drug delivery vehicle, and retain the biological activity when tested in an in vitro model system (Milella et al. 2001).

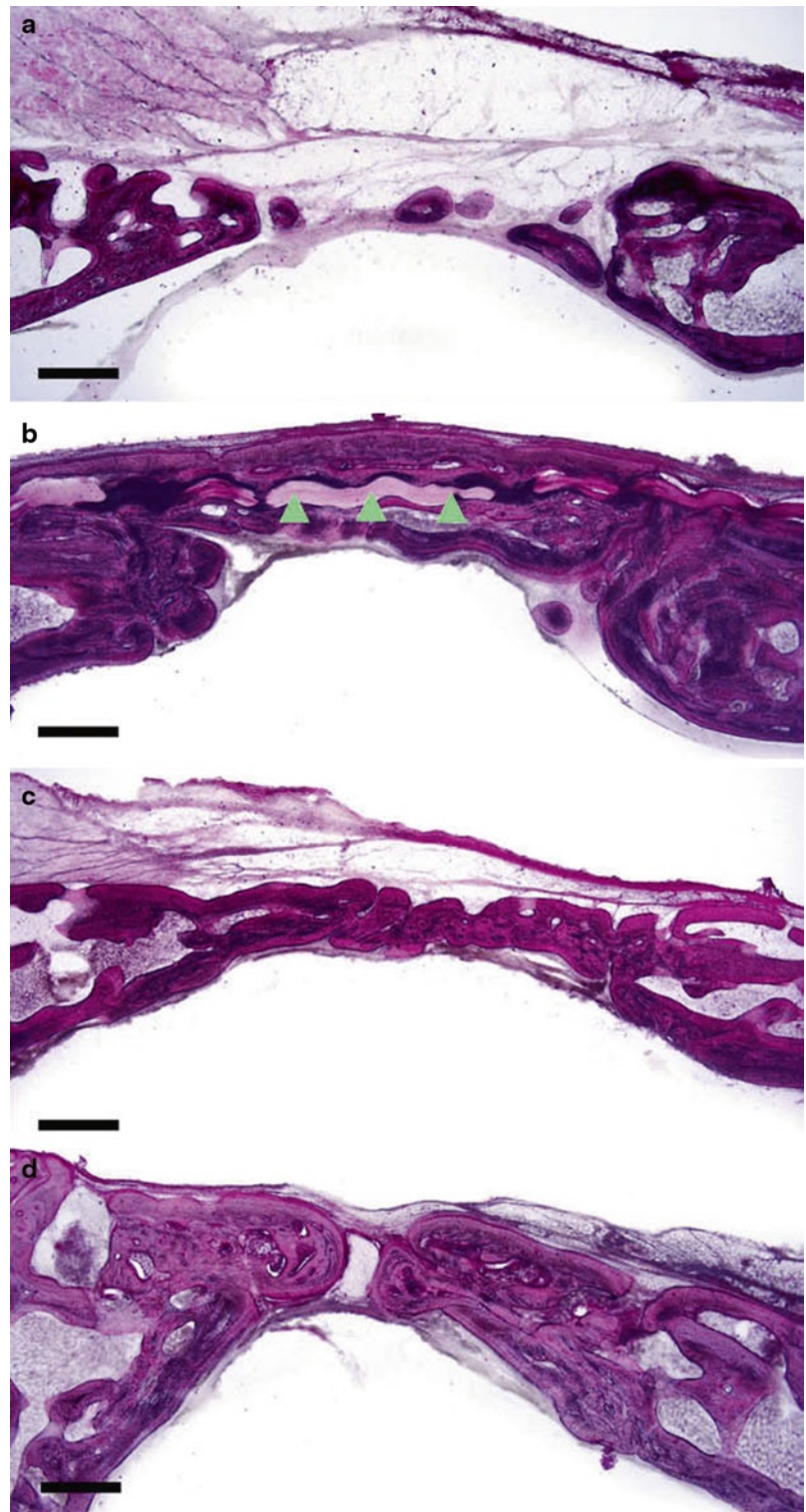
Platelet-derived growth factor-BB (PDGF-BB) was incorporated into porous poly(L-lactide) (PLLA) membranes with an aim of improving early bone healing in guided tissue regeneration (GTR) therapy. Porous PDGF-BB loaded membranes were fabricated by coating PDGF-BB-dissolved PLLA methylene chloride–ethyl acetate solutions on polyglycolic acid (PGA) meshes. Release of PDGF-BB could be controlled by adding bovine serum albumin that may provide porous diffusion channels for PDGF-BB release and by varying initial loading content of PDGF-BB. Biologic activity of PDGF-BB in the membranes was ascertained by fibroblast chemotaxis. PDGF-BB

loaded membranes maintained proper degradation property for periodontal application. PDGF-BB loaded membrane markedly increased new bone formation in rat calvarial defects, and completed bony reunion after 2 weeks of implantation period (Park et al. 1998).

Recently, *hyaluronic acid* (HA) and modified HA have been used for various medical applications, such as drug delivery and tissue engineering. Park et al. (2009) developed a novel biocompatible and degradation-controlled HA-poly(lactic-co-glycolic acid) (HA-PLGA) for the applications to periodontal barrier membranes. The degree of HA modification with adipic acid dihydrazide (adh) could be increased up to 85 mol% in the mixed solvent of water and ethanol. Highly modified HA-ADH appeared to be soluble in dimethyl sulfoxide with an enhanced stability to enzymatic degradation by hyaluronidase. When HA-PLGA was blended with PLGA in chloroform, amphiphilic biphasic films were obtained with hydrophilic HA and hydrophobic PLGA layers. According to in vitro degradation tests in PBS, HA-PLGA/PLGA (weight ratio of 1/2) film degraded relatively slowly compared to PLGA film and HA coated PLGA film. Among four different samples of a control, OSSIX™ membrane, PLGA film and HA-PLGA/PLGA film implanted to cover the calvarial critical size bone defects in SD rats, HA-PLGA/PLGA film resulted in the most effective bone regeneration followed by OSSIX™ membrane and PLGA film. The regenerated bone covered 63.1% of the bone defect area in 12 weeks (Fig. 1.40). The results might be ascribed to the biocompatible and degradation controlled characteristics of HA-PLGA/PLGA film with an enzymatic stability up to 8 weeks in the bone defect area. The novel biphasic HA-PLGA/PLGA film will be investigated further as a periodontal barrier membrane for clinical applications (Park et al. 2009).

Platelet-rich plasma (PRP) has been applied and studied in the field of bone grafting in implantology, oral surgery and periodontics. The mechanisms of PRP biological action have been poorly studied and are not well understood at the cellular and molecular level. Nonetheless, it is generally accepted that a variety of growth factors from platelets in PRP preparations are released upon its topical application, stimulating cell proliferation and attachment. PRP-coated membranes showed greater periodontal ligament (HPDL) and human gingival (HG) fibroblasts cell attachment compared to non-coated membranes. The cells showed

Fig. 1.40 Photomicrographs of the calvarial critical size bone defect regions in SD rats after bone regeneration for 12 weeks: **(a)** control, **(b)** OSSIX™ membrane, **(c)** poly(lactic-co-glycolic acid) (PLGA) film, and **(d)** PLGA grafted hyaluronic acid (HA-PLGA)/PLGA (1/2 weight ratio) blend film, respectively (scale bar: 1 mm) (Park et al. 2009. Reprinted with permission from Elsevier)



increased cytoplasmic extensions and appeared more intertwined around individual fibers of the membranes compared to uncoated control membranes. HPDL fibroblasts and HG fibroblasts have distinct reactions with different barrier membranes depending on both microstructure and composition of the membrane. It is clear that PRP has a significant impact on the way these cells attach to various barrier membranes by both increasing the amount and the quality of the attachment. However, how this increase in attachment is achieved and how well these in vitro results are translated into in vivo and actual clinical benefits still needs further investigation (Chang et al. 2007).

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Bone replacement grafts are widely used to promote bone formation and periodontal regeneration. Conventional surgical approaches, such as open flap debridement, provide critical access to evaluate and detoxify root surfaces as well as establish improved periodontal form and architecture; however, these surgical techniques offer only limited potential in restoring or reconstituting component periodontal tissues (Fig. 2.1). Bone grafting materials function, in part, as structural scaffolds and matrices for attachment and proliferation of anchorage-dependent osteoblasts. Multiple classification systems have been used to organize bone replacement grafts, which commonly include source (e.g., allograft), chemical composition (e.g., calcium phosphate) and physical properties (e.g., ceramic). Advances in material sciences, however, have increasingly blurred such boundaries between types of bone replacement grafts (Reynolds et al. 2010).

Bone replacement grafts (bone grafts and bone graft substitutes) provide a structural framework for clot development, maturation and remodeling that supports bone formation in osseous defects. Bone grafting materials also exhibit a variable capacity to promote the coordinated formation of bone, cementum and periodontal ligament (PDL) when placed and retained in periodontal defects. Bone grafting materials must possess the attributes of biocompatibility (lacking an immunogenic response) and osteoconductivity (providing a structure and surface topography that permit cellular attachment, proliferation and migration). Bone replacement grafts may also possess other properties that support osteogenesis (Reynolds et al. 2010). Ideal characteristics of a bone graft are: nontoxic, nonantigenic, resistant to infection, no root resorption or ankylosis, strong and resilient, easily adaptable, ready and sufficiently available, minimal

surgical procedure, stimulate new attachment and be able to trigger osteogenesis, cementogenesis and formation of a functional periodontal ligament (Rosenberg and Rose 1998; Nasr et al. 1999).

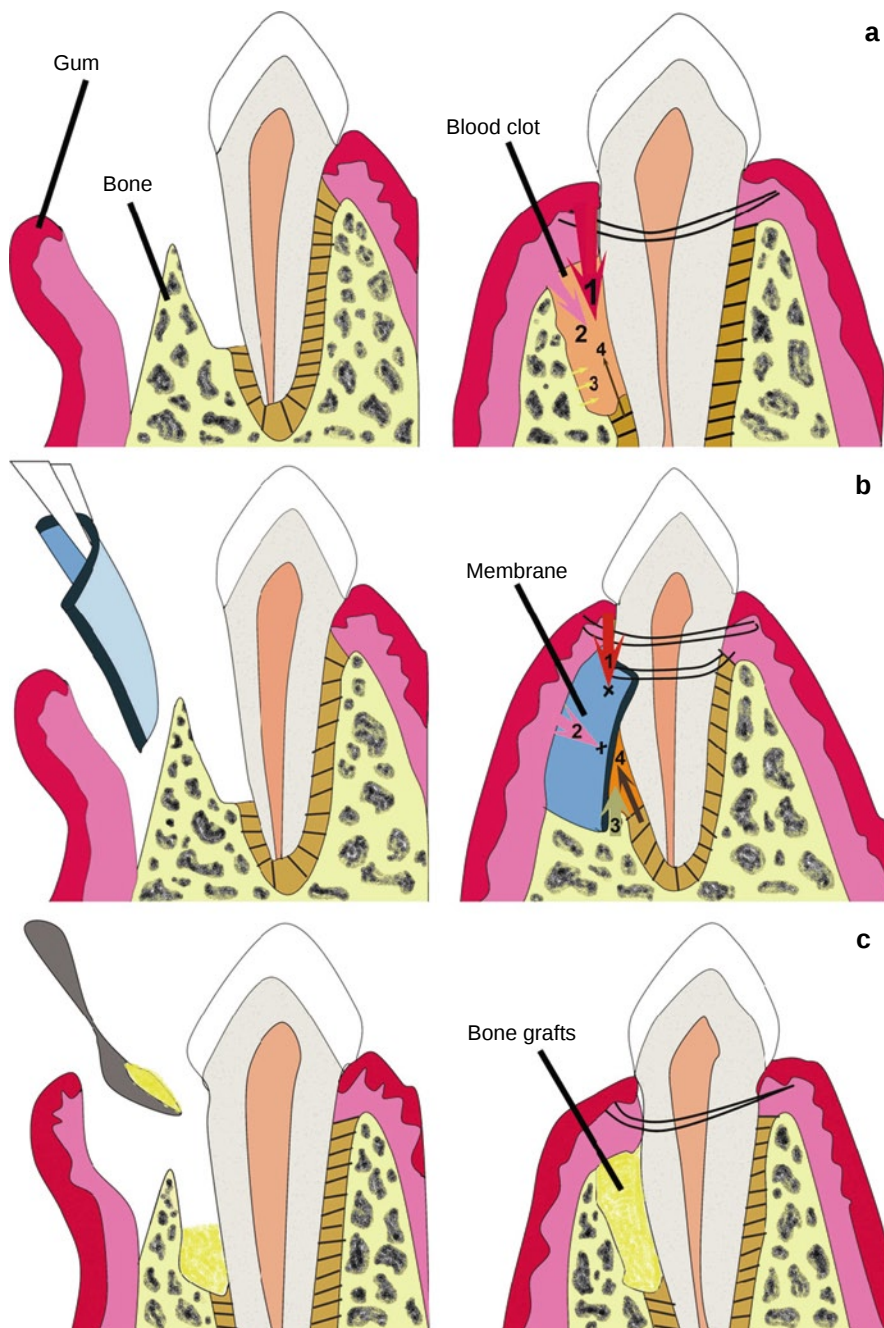
Osteogenic grafting materials, such as cancellous bone/bone marrow, contain living cells that are capable of differentiation and formation of bone. Osteoinductive grafting materials, such as demineralized bone matrix (DBM), provide a biologic stimulus (proteins and growth factors) that induces the progression of mesenchymal stem cells and other osteoprogenitor cells toward the osteoblast lineage (Reynolds et al. 2010). *Osteoinduction* is a process or a set of processes that stimulate the phenotypic conversion of progenitor cells within the healing wound to those that can form osseous tissue (Nasr et al. 1999). Most bone replacement grafts are osteoconductive, relatively inert filling materials and integrate with new bone. *Osteoconduction* defines the process that permits osteogenesis when cells already committed to bone formation are present in a closed environment (Nasr et al. 1999).

In general, bone replacement grafts can be categorized into autogenous, allograft, alloplast and xenograft sources.

2.1 Autogenous Grafts

Autogenous bone graft, which is harvested from the patient's own body, is considered ideal because of its osteoconductive and osteoinductive properties and because it contains a source of osteoprogenitor cells. It is still considered the gold standard by which other grafting materials are compared (Rosenberg and Rose 1998).

Fig. 2.1 Schematic diagrams of several techniques commonly used in periodontal surgery. (a) Open flap debridement (OFD) procedure involves the periodontal surgeon lifts the gum away from the tooth and surrounding bone, providing increased access for scaling and root planing. However, periodontal defects, if left empty after OFD, fill with the first cells to reach the area, that is, epithelial cells (1) and fibroblasts (2), after cell proliferation, which generates a core of fibro-epithelial tissues that attach to the root surface, hence bone (3) and periodontal ligament (4) regeneration are hampered. (b) Guided tissue regeneration (GTR) is a surgical procedure that utilizes a barrier membrane which is placed under the gum and over the remaining bone to prevent epithelial down-growth (1) and fibroblast transgrowth (2) into the wound space, thereby maintaining a space for true periodontal tissue regeneration (3 and 4). (c) The use of bone grafts is a surgical procedure that replaces missing bone with material from the patient's own body (autogenous bone) or an artificial, synthetic or natural substitute. Bone growth may be stimulated by the grafts and new bone fills the defect which may provide support for the tooth (Chen et al. 2010) (Reprinted with permission from Elsevier)



2.1.1 Intraoral Autografts

Intraoral autogenous bone grafts harvested from the maxillary tuberosity, edentulous alveolar areas, healing bony wound, extraction sites and mental and retro-molar areas (Nasr et al. 1999, Rosenberg and Rose 1998) (Fig. 2.2).

Several types of autogenous bone grafts can be used (Rosenberg and Rose 1998; Mellonig 1992):

- (a) *Cortical bone chips* – These are not used today because they are generally much longer particles $1,559.6 \times 183 \mu\text{m}$ and have a higher potential for sequestration (Zaner and Yukna 1984).

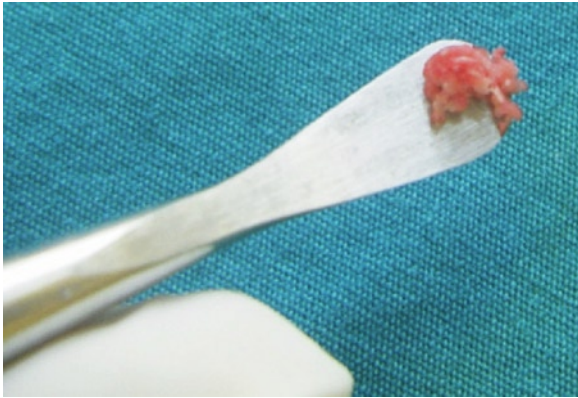


Fig. 2.2 The collected bone particles were carried by sterile periosteal elevator (Tezulas et al. 2009) (Reprinted with permission from Elsevier)

- (b) *Osseous coagulum* – This is made by harvesting intraoral bone with round burns, and then mixing it with blood (Robinson 1969; Jacobs and Rosenberg 1984). Early studies in monkeys showed that small particle size (100 μm) led to earlier and higher osteogenic activity than did the larger particles (Rivault et al. 1971). The disadvantages in using the osseous coagulum are the patient's inability to aspirate during the collecting process, the unknown quality of the collected bone fragments, and the material's fluidity (Diem et al. 1972).
- (c) *Blend of cortical and cancellous intraoral bone* – Bone blend is the combination of cortical and cancellous bone that is procured with a trephine or rongeurs, placed in an amalgam capsule, and triturated to the consistency of a slushy osseous mass. The final particle size is about $210 \times 105 \mu\text{m}$ (Zaner and Yukna 1984).

A technique based on the use of bone collectors for obtaining autogenous bone material, which allowed us to fill small bone defects, such as fenestrations and dehiscences, without having to involve a second (intraoral or extraoral) surgical area for obtaining autogenous bone was recently presented Blay et al. (2003).

Bone collected through bone filters appears to be sufficient for small regenerative procedures. Clinicians should bear in mind that the presence of bacterial pathogens is always shown with the use of bone collectors (Graziani et al. 2007). The stringent aspiration protocol, preoperative oral chlorhexidine rinse and antibiotic prophylaxis are important precautions to be implemented if collected bone articles will be implanted. With the

use of these methods, however, the risk of infectious complications remains. Therefore, decontamination processes are thought to only reduce microbial contamination (Tezulas and Dilek 2008). In order to overcome some of these problems, a newly developed piezoelectric device (Piezosurgery) has been recently introduced for different bone augmentation procedures. The main advantages of the piezoelectric device may be because of its modulated ultrasound microvibrations (29 kHz, ranging from 60 to 200 Hz) which should prevent damages to the adjacent soft tissues during osteotomy procedures. However, both the harvesting methods, piezoelectric device or conventional rotating drills, are not different from each other concerning their detrimental effect on viability and differentiation of cells growing out of autogenous bone chips derived from intraoral cortical sites (Chiriac et al. 2005).

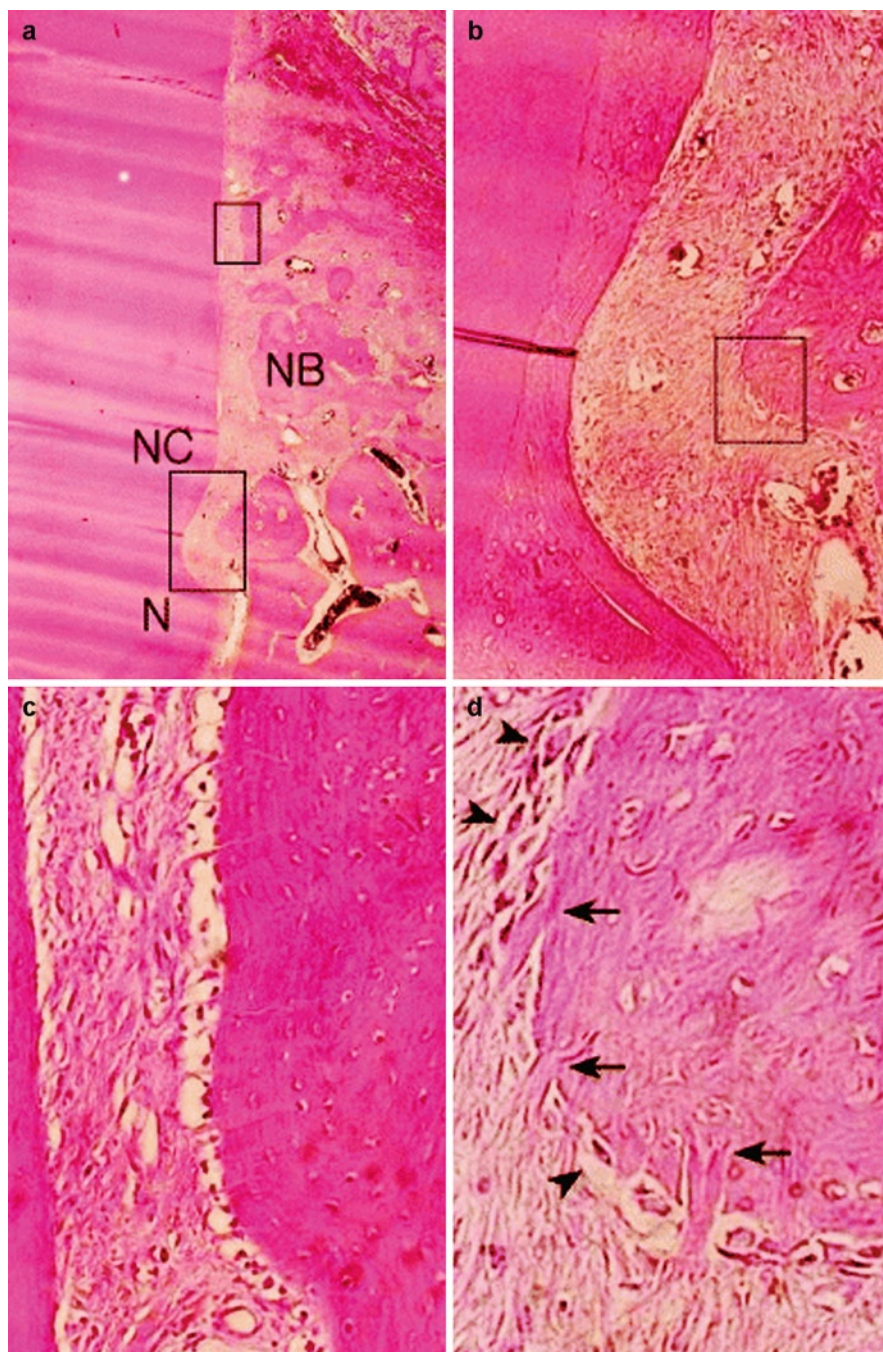
2.1.2 Extraoral Autografts

Extraoral autografts from iliac cancellous bone and marrow provide a great osteogenic potential, being able to induce cementogenesis, bone regeneration and Sharpey's fibers reattachment (Rosen et al. 2000).

Among the biomaterials, autogenous bone has been adopted as the gold standard because: (1) autograft bone includes cells participating in osteogenesis, (2) a tissue reaction is induced without inducing immunological reactions, (3) there is a minimal inflammatory reaction, (4) there is rapid revascularization around the graft particles and (5) a potential release of growth and differentiation factors sequestered within the grafts (Marx 1994; Kim et al. 2005). Histological studies show that intraoral autografts are able to form new connective tissue attachment when implanted in intra-bony defects (Froum et al. 1983). In baboons, Cochran et al. (2003) showed that the combination of enamel matrix derivative and autogenous bone represents a therapeutic combination that can be highly effective in stimulating significant amounts of periodontal regeneration (Fig. 2.3).

In perspective, autograft bone has been considered to yield a high osteogenic potential and has thus been used with the intent to improve outcomes of periodontal regenerative procedures (Dragoo and Sullivan 1973a; Froum et al. 1975a, 1975b, 1976; Hiatt and Schallhorn 1973; Ogawa et al. 1985; Renvert et al. 1985a;

Fig. 2.3 Periodontal healing in one-wall intrabony defects in dogs following implantation of autogenous bone. (a) Photomicrograph of site receiving autologous bone showing new bone (NB) and cementum (NC) formation in the notch area (N) (H-E, original magnification $\times 20$). The higher magnifications show the oblique or perpendicular collagen fiber arrangement. (b) A thick layer of new cementum with an oblique or perpendicular collagen fiber arrangement was observed in the notch area (H-E, original magnification $\times 100$). (c) A well-organized periodontal ligament exhibiting a thinner new cementum layer was observed more coronally (H-E, original magnification $\times 200$). (d) Newly formed bone trabeculae were lined with osteoblast-like cells (arrowhead) and exhibited perpendicularly oriented fibers (arrows) (H-E, original magnification $\times 400$) (Kim et al. 2005) (Reprinted with permission from John Wiley & Sons)



Kim et al. 2005) (Fig. 2.4). Combined therapies using autogenous bone grafts with guided tissue regeneration (GTR) (Orsini et al. 2001, 2008; Camelo et al. 2001; Nygaard-Østby et al. 2008, 2010; Lindfors et al. 2010), Emdogain (Leung and Jin 2003; Trombelli et al. 2006; Guida et al. 2007; Yilmaz et al. 2010), platelet-rich plasma (Czuryszkiewicz-Cyrana and Banach 2006) and

autogenous periodontal ligament graft (Shirmohammadi et al. 2009) in the treatment of intrabony, furcation defects or peri-implantitis have been investigated.

In the review performed by Trombelli et al. (2002), only one parallel-arm trial (Movin and Borring-Møller 1982) comparing autogenous bone grafts to open flap debridement procedure was selected. The results

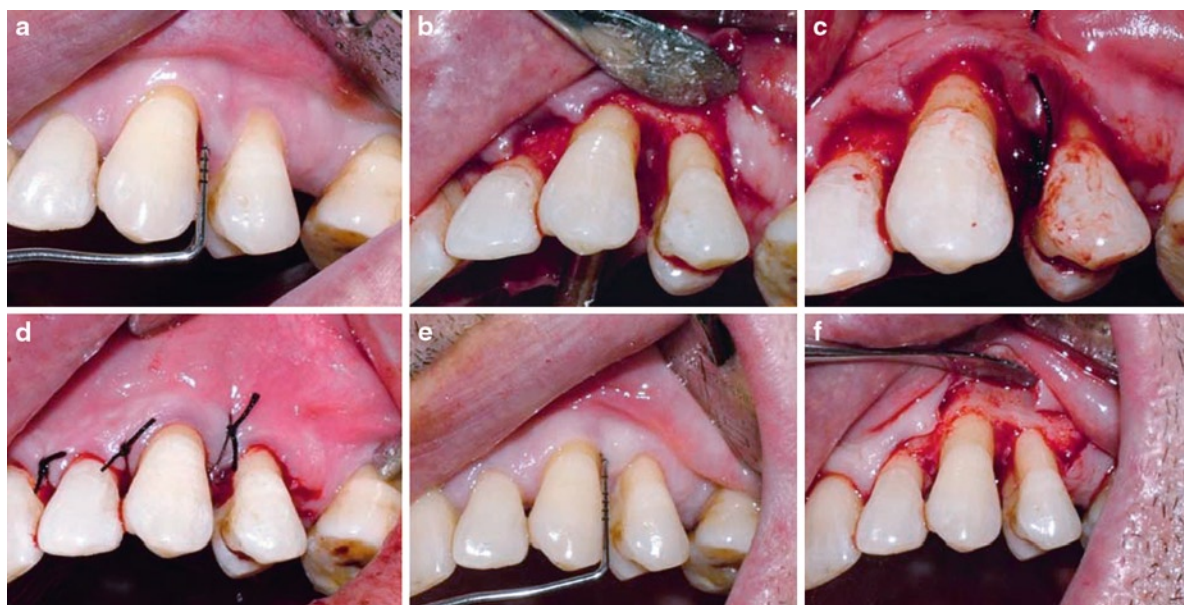


Fig. 2.4 Case from autogenous bone graft group: (a) clinical appearance of the intrabony defect at tooth 23 at the time of surgery, (b) appearance of the defect, (c) placement of the autog-

enous bone graft, (d) suturing, (e) clinical appearance at 6 months posttreatment and (f) reentry (Shirmohammadi et al. 2009) (Reprinted with permission from Springer)

indicated a greater clinical attachment level (CAL) gain for grafted group (CAL gain: 3.2 mm, SD 0.5) compared with controls (CAL gain: 2.0 mm, SD 0.8). The difference in CAL gain between groups (1.20 mm, SE 0.39) was not statistically significant. In the systematic review by Reynolds et al. (2003), two studies were included: Froum et al. (1976) and Renvert et al. (1985b). Autogenous bone treatment resulted in significantly greater clinical attachment level gain (weighted mean difference: 0.72 mm, SD 1.82) and bone fill (weighted mean difference: 1.62 mm, SD 1.53) for autogenous bone compared to open flap debridement (Table 2.1).

Although autograft procedures fulfill many of the characteristics of an ideal bone graft material, autografts are more invasive due to the additional surgical manipulations required to obtain donor tissue, and are limited by the relatively small quantity of bone that can be obtained from such techniques. These procedures also have been associated with postoperative root resorption. As a result, autografts may not be routinely practical in severe periodontitis cases involving multiple teeth and severe defects (Cohen et al. 1994). Root resorption and ankylosis in human after the transplantation of fresh iliac marrow and cancellous bone has been reported (Dragoo and Sullivan 1973b; Schallhorn and Hiatt 1972).

2.2 Allografts

The allografts are obtained from other individuals of the same species but disparate genotype. They include freeze-dried bone allografts (FDBA) and demineralized freeze-dried bone allograft (DFDBA). Bone allograft is the most frequently used alternative to autogenous bone for bone grafting procedures in the USA (Reynolds et al. 2010).

The two types of allografts work by different mechanisms. FDBA provides an osteoconductive scaffold and elicits resorption when implanted in mesenchymal tissues. DFDBA also provides an osteoconductive surface. In addition, it provides a source of osteoinductive factors. Therefore, it elicits mesenchymal cell migration, attachment and osteogenesis when implanted in well-vascularized bone, and it induces endochondral bone formation when implanted in tissues that would otherwise not form bone (Committee on Research, Science and Therapy of the American Academy of Periodontology 2001).

Under FDA regulations, facilities engaged in procuring and processing human tissues for transplantation must ensure that specified minimum medical screening and infectious disease testing have been performed, and that records exist and are maintained to

Table 2.1 Mean differences (in mm) in clinical outcomes between reconstructive procedure and control (open flap debridement) procedures as assessed in systematic reviews

Reconstructive procedure	Systematic reviews	Outcome	No. of studies	Weighted mean difference (mm)	95% CI (SD) [SE]	P-value for difference	P-value for heterogeneity
GTR	Murphy and Gunsolley (2003)	CAL change	18	1.15	N/A	<0.0001	N/A
		PD change	15	1.04	N/A	<0.0001	<0.01
	Needleman et al. (2001)	CAL change	10	1.11	0.63, 1.59	*	<0.001
		PD change	5	0.80	0.14, 1.46	*	0.04
		Bone gain at re-entry	3	1.39	1.08, 1.71	*	0.65
GTR + bone substitutes	Needleman et al. (2001)	CAL change	2	1.25	0.89, 1.61	*	0.91
		PD change	2	1.24	0.89, 1.59	*	0.85
		Bone gain at reentry	1	3.37	3.14, 3.61	*	–
Autogenous bone graft	Trombelli et al. (2002)	CAL change	1	1.20	[0.39]	>0.20	–
	Reynolds et al. (2003)	CAL change	3	0.72	(1.82)	0.030	NS
		PD change	1	0.60	(1.35)	0.062	–
		Bone fill	2	1.62	(1.53)	0.058	≤0.004
Bone allograft	Trombelli et al. (2002)	CAL change	6	0.36	–0.16, 0.87	0.174	0.013
		PD change	6	0.41	0.16, 0.66	0.001	0.067
	Reynolds et al. (2003)	CAL change	11	0.44	(2.25)	0.008	NS
		PD change	9	0.43	(2.25)	0.032	NS
		Bone fill	12	1.06	(1.97)	<0.0001	NS
Dentalallograft	Trombelli et al. (2002)	CAL change	1	0.80	[0.38]	>0.50	–
Coralline calcium carbonate	Trombelli et al. (2002)	CAL change	4	0.90	0.53, 1.27	<0.001	0.104
		PD change	4	0.04	–1.78, 1.87	0.962	<0.001
	Reynolds et al. (2003)	CAL change	4	0.91	(1.94)	0.004	NS
		PD change	4	0.09	(2.16)	0.886	NS
		Bone fill	3	2.21	(1.82)	<0.0001	NS
Bioactive glass	Trombelli et al. (2002)	CAL change	4	1.04	0.31, 1.76	0.005	0.024
		PD change	4	0.60	0.20, 1.00	0.003	0.684
	Reynolds et al. (2003)	CAL change	4	1.05	(1.89)	0.022	NS
		PD change	4	0.71	(2.22)	0.018	NS
		Bone fill	4	1.61	(1.47)	0.086	0.006

Table 2.1 (continued)

Reconstructive procedure	Systematic reviews	Outcome	No. of studies	Weighted mean difference (mm)	95% CI (SD) [SE]	P-value for difference	P-value for heterogeneity
Porous/nonporous hydroxyapatite	Trombelli et al. (2002)	CAL change	4	1.40	0.64, 2.16	<0.001	0.013
		PD change	5	0.98	0.67, 1.29	<0.000	0.070
	Reynolds et al. (2003)	CAL change	4	1.20	(2.22)	0.003	NS
		PD change	6	0.74	(2.12)	0.030	NS
		Bone fill	5	1.58	(1.77)	<0.000	≤0.04
PMMA-PHEMA	Trombelli et al. (2002)	CAL change	1	0.90	[0.22]	0.001	–
		PD change	1	0.90	N/A	0.003	–
	Reynolds et al. (2003)	Bone fill	1	1.26	N/A	0.001	–
Polylactic acid granules	Trombelli et al. (2002)	CAL change	1	–1.45	N/A	N/A	–
		PD change	1	–1.60	(0.55)	N/A	–
	Reynolds et al. (2003)	Bone fill	1	–0.28	N/A	0.519	–
Enamel matrix proteins	Trombelli et al. (2002)	CAL change	5	1.33	0.78, 1.88	<0.000	<0.001
		PD change	5	1.60	0.59, 2.62	0.002	<0.001
	Esposito et al. (2003)	CAL change	8	1.31	0.84, 1.78	<0.001	<0.001
		PD change	8	0.96	0.50, 1.41	<0.001	0.002
		Radiographic bone level	1	2.0	0.88, 3.12	<0.001	–

Source: Trombelli et al. (2005). Reprinted with permission from John Wiley & Sons

CAL clinical attachment level, CI confidence interval, GTR guided tissue regeneration, PD probing depth, PMMA-PHEMA polymethylmethacrylate and polyhydroxyethylmethacrylate, SD standard deviation, SE standard error, NS not significant

*P values are not given for Needleman et al. (2001) because the 95% confidence interval was reported

document screening and testing for each human tissue. The American Association of Tissue Banks also sets standards, inspects facilities and accredits tissue banks in North America (Reynolds et al. 2010).

Both FDBA and DFDBA materials are widely used in periodontal therapy and there are no reports of disease transmission during the 30-year history of using freeze-dried bone allografts. Most bone banks adhere to the guidelines of the American Association of Tissue Banks (AATB) with respect to procurement, processing and sterilization of bone grafts (Centers for Disease Control and Prevention 2010). The AATB advocates excluding collection of bone under the following circumstances:

1. Donors from high-risk groups, as determined by medical testing and behavioral risk assessments
2. Donors test positive for HIV antibody by ELISA
3. Autopsy of donor reveals occult disease

4. Donor bone tests positive for bacterial contamination
5. Donor and bone test positive for hepatitis B surface antigen (HBsAG) or hepatitis C virus (HCV)
6. Donor tests positive for syphilis

The net result of human bone allograft processing is an exponential reduction in the potential for graft contamination, disease transfer or both. Initial processing of human bone allografts typically involves stripping the bone of its soft tissue and sectioning it into smaller, more manageable pieces of approximately 5 mm in diameter. After the technician has cleansed the bone of soft tissue and has decontaminated it, proprietary processing takes place via one of many paths; some tissue-processing techniques involve liquid nitrogen freezing followed by lyophilization, whereas others involve repetitious wash treatments with solvents such as acetone. Although different, these procedures

produce similar results by eliminating nearly all of the moisture content from the bone, reducing antigenicity and facilitating extremely lengthy shelf storage at room temperature. If the final product is a freeze-dried bone allograft (FDBA), processing technicians reduce the processed bone to a particle size usually ranging between 250 and 750 μm , resample it for quality control, package it in sterile containers and may terminally sterilize it with low-dose γ irradiation (Table 2.2). If the final product is intended to be a demineralized freeze-dried bone allograft (DFDBA), the technician typically immerses the bone in a hydrochloric acid bath for various lengths of time to demineralize the bone by removing calcium. After acid treatment, the technician washes the newly demineralized bone allograft in

various buffer solutions to remove residual acid, rinses it to remove the buffer and terminally processes it in a fashion similar to that used for FDBA (Table 2.3).

Table 2.2 Steps in the processing of freeze-dried bone allograft (Holtzclaw et al. 2008) (Copyright © 2008 American Dental Association. All rights reserved. Reproduced by permission)

<i>Processing step 1. Soft tissue stripping</i> The technician removes residual muscle, tendon, ligament and so forth.
<i>Processing step 2. Initial size reduction</i> The technician reduces the bone to pieces of approximately 5 mm diameter for easier processing.
<i>Processing step 3. Initial cleansing and decontamination</i> The technician flushes, agitates, centrifugates or does all of these to the bone particles using various solutions such as saline, acetone, ethanol or hydrogen peroxide to remove residual bioburden and reduce antigenicity.
<i>Processing step 4. Microbiological treatment</i> The technician treats the bone particles with antimicrobial, antimycotic and antifungal solutions.
<i>Processing step 5. Freezing</i> The technician freezes the bone particles in liquid nitrogen of a temperature as low as -80°C .
<i>Processing step 6. Dehydration</i> The technician lyophilizes or treats the bone particles with repetitive solvent washes to eliminate moisture content and reduce antigenicity.
<i>Processing step 7. Secondary size reduction</i> The technician reduces the bone particles to final particulate sizes ranging between approximately 250 and 750 μm .
<i>Processing step 8. Packaging</i> The technician packages the bone allograft in sterile containers.
<i>Processing step 9. Terminal sterilization</i> The technician applies low-dose γ irradiation at low temperatures to ensure sterility (sterility assurance level, 10–6).

Table 2.3 Steps in the processing of demineralized freeze-dried bone allograft (Holtzclaw et al. 2008) (Copyright © 2008 American Dental Association. All rights reserved. Reproduced by permission)

<i>Processing step 1. Soft tissue stripping</i> The technician removes residual muscle, tendon, ligament and so forth.
<i>Processing step 2. Initial size reduction</i> The technician reduces the bone to pieces of approximately 5 mm diameter for easier processing.
<i>Processing step 3. Initial cleansing and decontamination</i> The technician flushes, agitates, centrifugates or does all of these to the bone particles using various solutions such as saline, acetone, ethanol or hydrogen peroxide to remove residual bioburden and reduce antigenicity.
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<i>Processing step 5. Freezing</i> The technician freezes the bone particles in liquid nitrogen of a temperature as low as -80°C .
<i>Processing step 6. Dehydration</i> The technician lyophilizes or treats the bone particles with repetitive solvent washes to eliminate moisture content and reduce antigenicity.
<i>Processing step 7. Secondary size reduction</i> The technician reduces the bone particles to final particulate sizes ranging between approximately 250 and 750 μm .
<i>Processing step 8. Demineralization</i> The technician immerses the allograft particles in a hydrochloric acid bath at concentrations ranging from 0.5 to 0.6 normal for various lengths of time.
<i>Processing step 9. Buffering</i> The technician again immerses the demineralized allograft particles in buffering solution to remove residual acid.
<i>Processing step 10. Final rinse</i> The technician again rinses the demineralized allograft with various solutions (e.g., distilled water) to remove residual buffer solution.
<i>Processing step 11. Packaging</i> The technician packages the bone allograft in sterile containers.
<i>Processing step 12. Terminal sterilization</i> The technician applies low-dose γ irradiation at low temperatures to ensure sterility (sterility assurance level 10–6).

Thus, rigorous donor screening and aseptic proprietary processing programs have rendered the use of human bone allografts safe and effective as a treatment option (Holtzclaw et al. 2008).

2.2.1 Freeze-Dried Bone Allografts (FDBA)

FDBA, which is not demineralized, works primarily through *osteoconduction*, a process in which the graft does not activate bone growth, but instead acts like a scaffold for the patient's own natural bone to grow onto and within. Over time, the graft is resorbed and replaced by new bone (Rosenberg and Rose 1998; Nasr et al. 1999).

FDBA is also a useful material clinically. There have been no reports of virus contamination or acquired pathology from FDBA, although this material is in wide use clinically (Committee on Research, Science and Therapy of the American Academy of Periodontology 2001). It was used in the treatment of three-wall intrabony defects adjacent to implants in dogs (Choi et al. 2010), in maxillary sinus-augmentation procedures (Koleran et al. 2008), in alveolar ridge augmentation alone (Fagan et al. 2008) or associated with platelet-rich plasma (Kassolis et al. 2000), in the treatment of periodontal defects, alone (Nevins et al. 2007; Laurell et al. 1998), combined with enamel matrix derivative (Rosen and Reynolds 2002) or with barrier membrane (Rosen and Reynolds 2001). FDBA can be combined with antimicrobial therapy, and has been used with tetracycline to regenerate experimental defects in baboons (Drury and Yukna 1991) or during treatment of localized juvenile periodontitis (Evans et al. 1989; Mabry et al. 1985). FDBA may be regarded as a graft material lacking clinically significant antigenicity (Quattlebaum et al. 1988).

2.2.2 Demineralized Freeze-Dried Bone Allografts (DFDBA)

Demineralization of a bone allograft exposes bone morphogenetic proteins within the bone matrix. These inductive proteins induce a cascade of events leading to cellular differentiation and the formation of bone

through osteoinduction by inducing pluripotent stem cells to differentiate into osteoblasts (Mellonig et al. 1992; Nasr et al. 1999).

It is important to evaluate the methods, procurement, processing and particle size of the demineralized freeze-dried bone allograft used in a study. When DFDBA is used in particulate form, *particle size appears to be an important variable in the success of DFDBA as a bone-inductive material*. Particles in the range of 125–1,000 μm possess a higher osteogenic potential than do particles below 125 μm . Optimal particle size appears to be between 100 and 300 μm . This may be due to a combination of surface area and packing density. Very small DFDBA particles elicit a macrophage response and are rapidly resorbed with little or no new bone formation. Tissue banks providing DFDBA for dental use will usually have this graft material in various particle sizes, and the range from 250 to 750 μm is the most frequently available (Committee on Research, Science and Therapy of the American Academy of Periodontology 2001). Schwartz et al. (1996) had shown a wide variation in commercial bone bank preparation of demineralized freeze-dried bone allograft and the ability to induce new bone formation. Particle size before implantation correlated with particle size after implantation. However, particle size did not correlate with ability to induce bone. The results show that commercial DFDBA differs in both size and ability to induce new bone formation, but that the two are not related.

Donor variability, however, also limits the predictability of DFDBA as an osteoinductive material (Boyan et al. 2006). The ability to induce bone appears to be age dependent, with DFDBA from older donors being less likely to have strong bone-inducing activity (Schwartz et al. 1998a).

The degree of DFDBA demineralization varies between tissue banks and may also affect clinical regeneration. A 2% residual calcium level in DFDBA has been shown to result in the highest alkaline phosphatase activity levels in cultured human periosteal cells and is optimally osteoinductive or osteoconductive for new bone formation (Herold et al. 2002).

Histological studies in humans performed by Bowers et al. (1989a, 1989b) revealed the formation of new attachment apparatus in intrabony defects grafted with DFDBA. At this time, demineralized freeze-dried bone allograft remains the only bone replacement graft proven to result in periodontal regeneration in a

controlled human histological study and is recognized in the consensus report by the 1996 World Workshop in Periodontics to fulfill all criteria considered for the promotion of periodontal regeneration (Nasr et al. 1999). The ability of demineralized bone to induce new bone formation in soft tissues and to enhance bone formation in osseous tissues is believed to be due to the content and diffusibility of bone morphogenetic proteins (BMPs) present in the material (Lohmann et al. 2001). The BMPs and other growth factors and cytokines interact with mesenchymal stem cells or undifferentiated osteogenic precursors in the host tissue, causing them to differentiate into bone-forming cells (Li et al. 2000; Lohmann et al. 2001; Schwartz et al. 1998b). Several other growth factors were identified: FGFa (fibroblast growth factor), IGF-I (insulin-like growth factor-I), TGF- β 1 (transforming growth factor-beta1), VEGF (vascular endothelial growth factor) and PDGF (platelet-derived growth factor) (Wildemann et al. 2007).

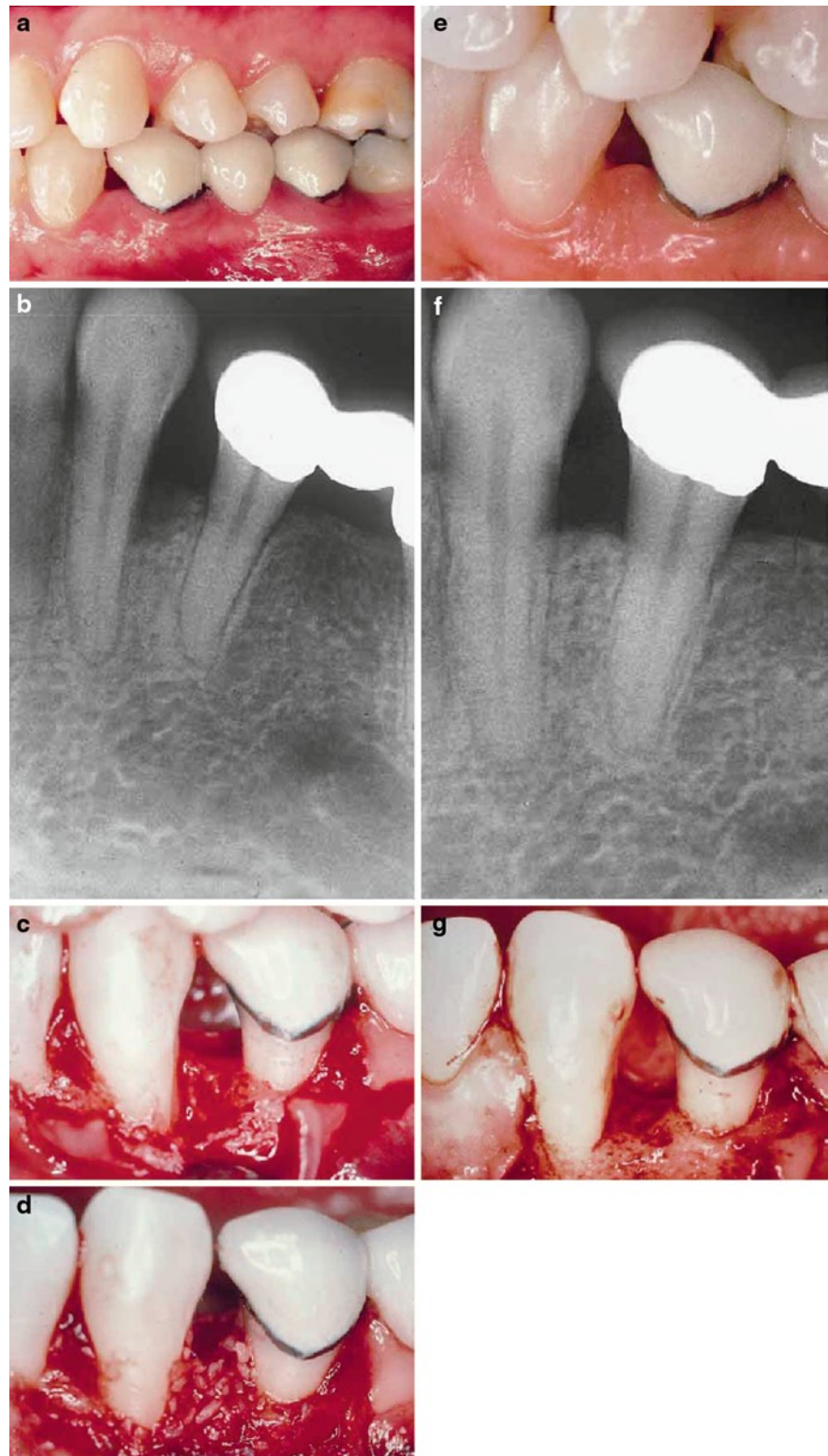
Six studies (Altieri et al. 1979; Blumenthal and Steinberg 1990; Borghetti et al. 1993; Flemmig et al. 1998; Masters et al. 1996; Yukna et al. 1998) comparing *bone allograft with open flap debridement* (OFD) procedure were included and pooled for meta-analysis by Trombelli et al. (2002). The results showed a limited, not statistically significant, greater CAL gain for grafted group with respect to OFD. Weighted mean difference between grafted and control was 0.36 mm (95% CI: -0.16 to 0.87; Q-test for heterogeneity, 14.40 (df=5), $P=0.01$). A significantly greater probing depth reduction was reported for bone allograft treatment: 0.41 mm (95% CI: 0.16–0.66) compared to OFD. Meta-analysis performed by Reynolds et al. (2003) on 12 studies (Altieri et al. 1979; Blumenthal and Steinberg 1990; Borghetti et al. 1993; Brown et al. 1998; Flemmig et al. 1998; Mabry et al. 1985; Masters et al. 1996; Meadows et al. 1993; Mellonig 1984; Mora and Ouhayoun 1995; Schrad and Tussing 1986; Yukna et al. 1998) showed a greater clinical attachment level gain for the grafted group with respect to OFD. Weighted mean difference between graft and control was 0.44 mm (SD 2.25). Adjunctive defect fill amounted to 1.06 mm (SD 1.97) with the use of bone allograft. A significantly greater probing depth reduction was reported for bone allograft treatment 0.43 mm, SD 2.25 compared to OFD (Fig. 2.5).

It may be possible to enhance the amount of bone fill obtained with bone replacement grafts such as DFDBA by combining regenerative therapies (Hanes 2007). *The use of replacement grafts (DFDBA) in an aim to improve the results of GTR therapy* for the treatment of intrabony or mucogingival defects was evaluated by several studies (Chen et al. 1995; Trejo et al. 2000; Lamb et al. 2001; Duval et al. 2000; Wang et al. 2002; Couri et al. 2002; Bowers et al. 2003; Kimble et al. 2004; Aichelmann-Reidy et al. 2004; Kothiwale et al. 2009). In studies where demineralized freeze-dried bone has been combined with barriers of cross-linked bovine collagen or expanded polytetrafluoroethylene membranes, the results tend to indicate that combined therapy is also a successful therapeutic approach (Rosen et al. 2000).

Recent studies suggest that the addition of enamel matrix derivative (EMD) to demineralized freeze-dried bone allograft may enhance osteoinduction (Boyan et al. 2000, 2006; Rosen and Reynolds 2002; Gurinsky et al. 2004; Harris et al. 2007; Hoidal et al. 2008; Aspriello et al. 2010). Venezia et al. (2004) concluded that EMD combination with allograft materials may be of additional benefit but still needs to be further investigated. Emdogain is composed primarily of amelogenin and other proteins present in embryonic porcine tooth germs. It is possible that a trace component of Emdogain possesses osteoinductive properties. During embryonic development, the interaction of epithelial and mesenchymal tissues is critical for tissue morphogenesis. It was suggested that Emdogain ability to enhance the osteoinductivity of DFDBA is the result of its properties as a bioactive matrix. Emdogain also delayed the rate at which DFDBA was resorbed, suggesting that factors present in this complex agent might modulate bone remodeling in addition to bone formation (Boyan et al. 2006). In contrast with previous results, it was also reported that DFDBA combined with EMD compared to DFDBA alone in the treatment of intraosseous defects of chronic periodontitis provided no statistically significant improvement to the soft and hard tissue parameters measured (Hoidal et al. 2008).

Several authors have investigated the impact of growth factors to demineralized freeze-dried bone allograft (DFDBA) (Bowers et al. 1991; Danesh-Meyer et al. 2001; Mott et al. 2002; Markopoulou

Fig. 2.5 (a) Preoperative view of the mandibular left canine in a 39-year-old Caucasian woman. The patient is a nonsmoker. (b) Preoperative radiograph suggests that there is an intrabony lesion at the distal of the left premolar. (c) Full-thickness flap reflection reveals a combination two- and three-wall lesion. (d) After root planing, scaling, citric acid root surface treatment and intramarrow penetration, demineralized freeze-dried bone allograft is placed to fill the entire defect. (e) View of the site at 48 months postsurgery. There has been some loss in height of the interdental papilla. (f) Postoperative radiograph taken at 48 months suggests complete bone fill of the defect at the distal of the canine. (g) Reentry performed at 48 months postsurgery shows hard tissue fill of the defect. There has been a slight loss of facial bone on the cuspid (Rosen et al. 2000) (Reprinted with permission from John Wiley & Sons)



et al. 2003; Camelo et al. 2003; Papadopoulos et al. 2003; Nevins et al. 2003a, 2007; Dereka et al. 2006, 2009; ; Markou et al. 2009, 2010). It has been showed that addition of BMP-2 increased osteoinduction of DFDBA by almost 50% based on the bone induction score (Boyan et al. 2006). DFDBA contains BMP-2, but the amount seems to vary among individuals. Moreover, the formatting of BMP-2 within DFDBA is different from that of BMP-2 adsorbed to the surface of the graft particles. Studies suggest that the DFDBA particles must be resorbed for the BMP contained within the matrix to be released. DFDBA becomes, in effect, a time-release carrier for these factors. Surface-adsorbed BMP-2 is released in a burst and, as a result, has its greatest effects on cells present at the implant site. Thus, the two forms of the morphogen work on distinctly different cell populations, and the combined effect is additive, if not synergistic. Other factors present in DFDBA also may contribute to the overall tissue response (Boyan et al. 2006). The evaluation of the effect of a combination of BMPs with a graft/bone substitute relates to one single histomorphometric study in humans where the association of BMP-3 (osteogenin) and two different biomaterials (purified bovine collagen and DFDBA) has been evaluated (Bowers et al. 1991). Test treatments consisted of the association of BMP-3 with DFDBA or bovine collagen; control groups consisted of the grafts used alone. Histologic evaluation at 6 months indicated that osteogenin combined with DFDBA significantly enhanced regeneration of a new attachment apparatus and component tissues in a submerged environment. DFDBA plus osteogenin and DFDBA alone formed significantly more new attachment apparatus and component tissues than either the tendon-derived matrix plus osteogenin or the tendon-derived matrix alone in both submerged and nonsubmerged environments. There were no significant differences between the tendon-derived matrix plus osteogenin and the tendon-derived matrix alone in either the submerged or nonsubmerged environment (Bowers et al. 1991). The combination of BMPs and allograft is a promising step forward in improving allograft treatment. As the number of allograft procedures performed per year increases worldwide, the outcome for many could be influenced by the addition of BMPs. Still, safety concerns as well as availability

of allograft is a continuous source of inspiration for those investigating alternative bone sources (Blokhuis and Lindner 2008).

Osteoblast proliferation rates indicate that the in vitro supplementation of 2% residual calcium-DFDBA with the combination of insulin-like growth factor (IGF) and transforming growth factor- β (TGF- β), IGF and platelet-derived growth factor (PDGF), and PDGF and TGF- β significantly ($P \leq 0.05$) enhances murine osteoblast activity and proliferation at 7 days compared with the control containing no exogenous growth factors (Mott et al. 2002). In contrast with these reports and to the increase in osteoinduction seen when DFDBA is implanted with BMP-2 or Emdogain, PDGF and PRP reduced osteoinductivity by approximately 20%. The inhibitory effect of PDGF-BB on DFDBA-induced bone formation is concentration dependent and, at high concentrations, causes the chondrogenic phase of endochondral bone formation to persist. At low concentrations, PDGF-BB does not inhibit DFDBA activity and, in an orthotopic site where other osteogenic signals are present, its effect on mesenchymal cell proliferation may result in increased bone formation (Boyan et al. 2006). Nevins et al. (2003a) revealed a robust regeneration of a complete new attachment apparatus, including bone, periodontal ligament and cementum in human interproximal intrabony defects and molar Class II furcation lesions following the application of purified recombinant human platelet-derived growth factor BB (rhPDGF-BB) incorporated in demineralized freeze-dried bone allograft (DFDBA) (Nevins et al. 2003a). In a randomized, double-masked, clinical trial, Piemontese et al. (2008) compared platelet-rich plasma (PRP) combined with a demineralized freeze-dried bone allograft (DFDBA) to DFDBA mixed with a saline solution in the treatment of human intrabony defects. No statistically significant differences were observed in the hard tissue response between the two treatment groups, which confirmed that PRP had no effect on hard tissue fill or gain in new hard tissue formation. It was recently demonstrated that both PRP and PRP combined with DFDBA resulted in significant clinical and radiographic improvement in human periodontal endosseous defects at 6 and 12 months (Piemontese et al. 2008; Markou et al. 2009, 2010), and the addition of DFDBA to PRP did not significantly enhance the treatment outcome

(Markou et al. 2009). A recent randomized, double-masked, clinical trial compared platelet-rich plasma (PRP) combined with a demineralized freeze-dried bone allograft (DFDBA) to DFDBA mixed with a saline solution in the treatment of human intrabony defects. The test group (PRP + DFDBA) exhibited statistically significantly greater changes compared to the control group in probing depth reduction (4.3 ± 1.7 mm versus 2.6 ± 2.2 mm; $P < 0.05$) and clinical attachment gain (3.5 ± 2.1 mm versus 2.3 ± 2.4 mm; $P < 0.001$). No statistically significant differences were observed in the hard tissue response between the two treatment groups (Piemontese et al. 2008).

Demineralized bone matrix is produced by acid extraction of allograft. It contains type-1 collagen, non-collagenous proteins and osteoinductive growth factors. There are numerous demineralized bone matrix formulations based on refinements of the manufacturing process. They are available as freeze-dried powder, granules, gel, putty or strips. They have also been developed as combination products with other materials such as allogeneic bone chips and calcium sulfate granules (De Long et al. 2007). Demineralized bone matrix paste and putty are particulate demineralized bone matrices in a 2% or 4% hyaluronate carrier, respectively. When compared with demineralized freeze-dried bone allografts, all demonstrated similar favorable improvements in soft and hard tissue parameters in the treatment of human intraosseous defects (Bender et al. 2005).

Examples of commercially available products are Grafton® DBM (Osteotech, Inc. American Association of Tissue Banks), Grafton Plus® DBM Paste (Osteotech, Inc. American Association of Tissue Banks), Osseograft (Advanced Biotech Products (P) Ltd. India), Accell Connexus™ (Accell® technology + DBM particles + reverse phase medium for optimal handling) (IsoTis Orthobiologics/GenSci Regeneration Technologies), Accell® DBM100® (Accell® technology + DBM particles in putty) (IsoTis Orthobiologics/GenSci Regeneration Technologies), DBX® Demineralized Bone Matrix (Musculoskeletal Transplant Foundation, USA), Dynagraft putty (Gen-Sci, Regeneration Laboratories, CA) and Osteofil allograft bone paste (Regeneration Technologies, FL). Regenafile®, Altiva DBM Paste, BioSet™, RTI Allograft Paste and Osteofil® contain human demineralized freeze-dried bone allograft (DFDBA, also known as demineralized

bone matrix, DBM) in an inert porcine gelatin carrier. Regenaform®, Altiva DBM with cortical cancellous chips, BioSet™ IC, RTI Allograft Paste IC and Osteofil® ICM contain human DFDBA and human cortical-cancellous bone chips in an inert porcine gelatin carrier. Regenafile and Regenaform Frozen Allograft Paste should be stored frozen. It may be stored for 6 months at -20 to -40°C (conventional freezer) or up to 5 years if stored at -40°C or colder (see expiration date on label). Regenafile and Regenaform Frozen Allograft Paste must be warmed prior to use. Detailed instructions for warming the graft are included in the package insert that accompanies each graft (<http://www.exac.com/products/dental-biologics>).

The decision about which form of allograft to use should be based on the clinical condition of the site to be grafted. Because it is still mineralized, FDBA may have better physical characteristics. However, FDBA is not osteoinductive. Although no significant differences have been found clinically between FDBA and DFDBA in primarily intraosseous defects (Piattelli et al. 1996a; Rummelhart et al. 1989; Francis et al. 1995) in sites where regeneration may be more problematic, DFDBA may be a more appropriate choice (Committee on Research, Science and Therapy of the American Academy of Periodontology 2001). However, recent histological study suggested that FDBA may stimulate earlier, more rapid and more substantial new bone formation than DFDBA in a monkey jaw defect model system (Yukna and Vastardis 2005). A trend was observed toward greater improvement in clinical attachment level gain in advanced infrabony defects when EMD was combined with FDBA ($57.3\% \pm 9.4\%$) as compared to DFDBA ($49.1\% \pm 11.0\%$) (Rosen and Reynolds 2002).

2.3 Xenografts

Xenografts are grafts shared between different species. Currently, there are two available sources of xenografts used as bone replacement grafts in periodontics: bovine bone and natural coral. Both sources, through different processing techniques, provide products which are biocompatible and structurally similar to human bone. Recently, porcine bone xenografts have also been described. Xenografts are osteoconductive, readily

available and risk free of disease transmission. The latter point has been questioned with the discovery of bovine spongiform encephalopathy, particularly in Great Britain (Nasr et al. 1999).

2.3.1 Anorganic Bovine-Derived Bone Xenograft (BDX)

The BDX is a xenograft consisting of deproteinized, sterilized bovine bone with 75–80% porosity and a crystal size of approximately 10 μm in the form of cortical granules (Hürzeler et al. 1997; Piattelli et al. 1999). Regarding both the chemical and physical features, BDX is considered identical to the human bone (Berglundh and Lindhe 1997; Piattelli et al. 1999).

BDX has several characteristics and advantages when compared with freeze-dried demineralized bone: no donor site is required from the patients; unlimited supplies of the material are available; the material is easily handled and used as freeze-dried demineralized bone; and the results are predictable when good surgical principles are observed, a sterile environment is maintained and tissue is handled properly as recommended by the manufacturer (Callan et al. 1993).

Xenografts are bovine in origin and carry the theoretical risk of transmission of bovine spongiform encephalopathy (Precheur 2007). Several studies, however, indicate that the use of these materials does not carry a risk for transmitting bovine spongiform encephalopathy (BSE) to humans (Hönig et al. 1999; Wenz et al. 2001; Precheur 2007). Sogal and Tofe (1999) conducted an extensive review of current literature on the status of risk assessment of BSE transmission and two risk assessment models were identified as applicable to the present study. Risk assessment models developed by the German Federal Ministry of Health and by the Pharmaceutical Research and Manufacturers Association of America were applied to BGS. Results from the analyses conducted using both models showed that the risk of disease (BSE) transmission from BGS was negligible and could be attributed to the stringent protocols followed in sourcing and processing of the raw bovine bone used in the commercial product.

Several histological studies in animals and humans have revealed that BDX possesses excellent

osteoconductive properties (Berglundh and Lindhe 1997; Skoglund et al. 1997; Camelo et al. 1998, 2001; Hämmerle et al. 1998; Mellonig 2000; Zitzmann et al. 2001; Paolantonio 2002; Nevins et al. 2003a, 2003b; Sculean et al. 2003b, 2004a). The large-mesh interconnecting pore system facilitates angiogenesis and migration of osteoblasts (Orsini et al. 2005). Several histological studies have shown that BDX particles were surrounded for the most part by mature, compact bone. In some Haversian canals, it was possible to observe small capillaries, mesenchymal cells and osteoblasts in conjunction with new bone. No gaps were present at the interface between the BDX particles and newly formed bone, the BDX granules being interconnected by bridges of vital newly formed bone (Piattelli et al. 1999; Tadjoeidin et al. 2003). With time, BDX becomes integrated and subsequently replaced by newly formed bone (Berglundh and Lindhe 1997). In histological specimens retrieved after 18 months and 4 years, it was possible to observe the presence of osteoclasts in the process of resorbing the BDX particles and neighboring newly formed bone (Piattelli et al. 1999). It has been reported that BDX has a very low resorption rate (Valentini and Abensur 1997). However, how this biomaterial enhances osteoblast activity to promote bone formation is not completely understood. MicroRNAs (miRNAs) represent a class of small, functional, noncoding RNAs of 19–23 nucleotides that regulate the transcription of messenger RNAs (mRNAs) in proteins. The miRNA microarray technique was used to investigate translation regulation in an osteoblast-like cell line (MG63) exposed to Bio-Oss. It was showed that the vast majority of detected mRNAs were downregulated, including some homeobox genes (genes that regulate the morphogenesis of an entire segment of the body), such as *noggin* and *EN1*. An indirect positive effect was demonstrated on bone morphogenetic protein-4 (Palmieri et al. 2010). It was indicated that BDX and Perioglas act on different miRNAs. Globally, Perioglas causes activation of bone-forming signaling, whereas BDX also activates cartilage-related pathways (Annalisa et al. 2008).

Because all the protein is removed, this 100% crystalline hydroxyapatite grafting material is considered biocompatible (Cohen et al. 1994; Callan et al. 1993), is very well tolerated, and, until now, no adverse reactions such as allergies or rejection of the graft particles related to the material have been reported (Camelo et al. 1998; Richardson et al. 1999;

Camargo et al. 2000; Mellonig 2000; Zitzmann et al. 2001; Sculean et al. 2003, 2004a, 2004b, 2005a, 2005b, 2007b; Tonetti et al. 2004; Liñares et al. 2006). However, recently, Bannister and Powell (2008) presented an unusual reaction to BDX after a ridge augmentation by a mixture of autogenous bone and anorganic bovine bone with platelet-rich plasma and a bioabsorbable collagen membrane. Healing was uneventful, although after 4 months, upon flap reflection, no regenerated hard tissue was found. At the histologic examination, it was observed that the majority of the graft material demonstrated an intimate association with multinucleated foreign body-type giant cells. However, at the second procedure, the site was regrafted with an allograft/xenograft mixture and covered by a bioabsorbable collagen membrane and wound healing was uneventful.

BDX has demonstrated efficacy for:

- *Reconstruction of atrophied alveolar ridges* (Callan and Rohrer 1993; Artzi and Nemcovsky 1998; Zitzmann et al. 2001; Kotschy and Laky 2006; Esposito et al. 2006; Cardaropoli et al. 2005; Lang et al. 2007)
- *Around endosseous implants* (Berglundh and Lindhe 1997; Skoglund et al. 1997; Zitzmann et al. 1997; Hämmerle et al. 1998; Schlegel and Donath 1998; Juodzbalsys and Wang 2007)
- *Sinus elevation floor procedures* (Valentini et al. 1998, 2000; Valentini and Abensur 2003; Piattelli et al. 1999; Hallman et al. 2001; Wallace and Froum 2003; Orsini et al. 2005; Handschel et al. 2009; Bornstein et al. 2008; Beloti et al. 2008)
- *Healing of intrabony peri-implantitis defects* (Schou et al. 2003; Schwarz et al. 2006a, 2008, 2009; Esposito et al. 2008)
- *Periradicular surgery in large periapical lesions* (Dietrich et al. 2003; Taschieri et al. 2007)
- *Periodontal bone defects* where BDX has been evaluated when used alone (Hutchens 1999; Richardson et al. 1999; Scheyer et al. 2002; Scabbia and Trombelli 2004; Gupta et al. 2007), in association with membranes (Hutchens 1999; Camelo et al. 1998, 2001; Camargo et al. 2000; Simonpietri-C et al. 2000; Paolantonio et al. 2001; Pietruska 2001; Paolantonio 2002; Sculean et al. 2003, 2004a, 2005a, 2007b; Stavropoulos and Karring 2005;

Stavropoulos et al. 2003; Stavropoulos et al. 2004; Vouros et al. 2004; Tonetti et al. 2004; Reddy et al. 2006; Sakata et al. 2006; Liñares et al. 2006) and in combination with enamel matrix protein derivative (Lekovic et al. 2000; Lekovic et al. 2001; Scheyer et al. 2002; Velasquez-Plata et al. 2002; Zucchelli et al. 2003; Sculean et al. 2002b, 2004b)

A composite of autogenous bone and bone substitute is widely used in oral surgery procedures because it combines the osteogenic property of autogenous bone and the osteoconductive property of BDX (Fig. 2.6). It contains osteogenic cells and provides a scaffold and internal pores for bone cells to grow and remineralize to new bone. The relative proportions of autogenous bone and bone substitute vary (Pripatnanont et al. 2009). A systematic review recommended a proportion of 1:2 (Merkx et al. 2003). Pripatnanont et al. (2009) assessed new bone formation generated using three different proportions of autogenous bone (AB) and deproteinized bovine bone (BDX) in cortical skull defects in rabbits. The 1:2 group had significantly higher bone content than the 1:4 group. The proportions of 1:1 and 1:2 resulted in greater bone formation than the proportion of 1:4 (Pripatnanont et al. 2009). In deep intrabony defects treatment, at 12 months evaluation, the combined use of autogenous spongiosa with bovine-derived xenograft led to significantly greater gain of clinical attachment and hard tissue formation compared to the use of autogenous spongiosa alone (Zafropoulos et al. 2007).

Examples of commercially available bovine-derived bone replacement grafts are Bio-Oss® (Osteohealth Co., Shirley, NY), Bio-Oss Collagen® (Osteohealth Co., Shirley, NY), OsteoGraf/N® (CeraMed Dental, LLC, Lakewood, CO) and PepGen P-15® (Dentsply Friadent, Mannheim, Germany) (Sukumar and Drízhál 2008).

Bio-Oss® (Osteohealth Co., Shirley, NY) is a natural, nonantigenic, porous bone mineral matrix. It is produced by the removal of all organic components from bovine bone (Fig. 2.7). It is available in cancellous (spongiosa) and cortical granules and blocks. Bio-Oss undergoes a low-heat (3,000°C) chemical extraction process by which all organic components are removed, but maintains the natural architecture of bone (Richardson et al. 1999). This material is essentially

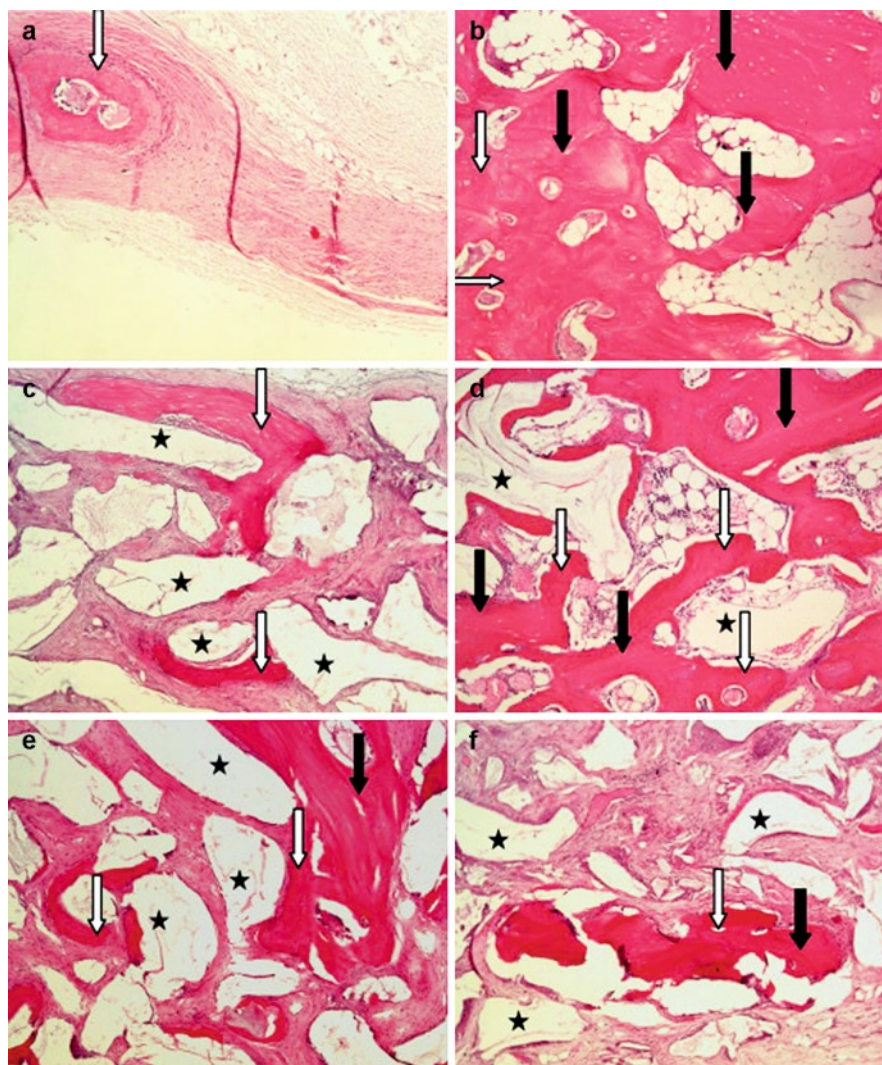


Fig. 2.6 Proportion of deproteinized bovine bone and autogenous bone affects bone formation in the treatment of calvarial defects in rabbits: (a) a critical size defect, (b) an autogenous bone chip, (c) BDX alone, (d) a composite graft of autogenous bone and BDX at 1:1 ratio, (e) a composite graft of autogenous bone and

BDX at 1:2 ratio, (f) a composite graft of autogenous bone to BDX at 1:4 ratio. *White arrows indicate new bone, black arrows indicate bone chips, black stars indicate BDX particles* (Hematoxylin and Eosin, original magnification $\times 10$) (Pripatnanont et al. 2009) (Reprinted with permission from Elsevier)

carbonate-containing apatite with few hydroxyl groups, and possesses a crystalline architecture and calcium:phosphate ratio similar to natural bone mineral in humans (Cohen et al. 1994). Due to its natural structure Bio-Oss is physically and chemically comparable to the mineralized matrix of human bone (Fig. 2.8). Chemically, Bio-Oss is a low crystalline apatite (crystallite size of approximately $100 \times 200 \times 500 \text{ \AA}$) with a

7% content of carbonate (Benke et al. 2001). The infrared spectra and X-ray diffraction patterns show a calcium content of $37.1 \pm 0.7\%$ and a phosphorous content of $17.8 \pm 0.5\%$, corresponding to a Ca-P ratio of 2.1 ± 0.1 (Jensen et al. 1996; Scabbia 2004 Sculean et al. 2007b).

Bio-Oss Collagen[®] (Osteohealth Co., Shirley, NY) consists of Bio-Oss Spongiosa granules (0.25–1 mm)



Fig. 2.7 Bio-Oss® (Osteohealth Co., Shirley, NY)

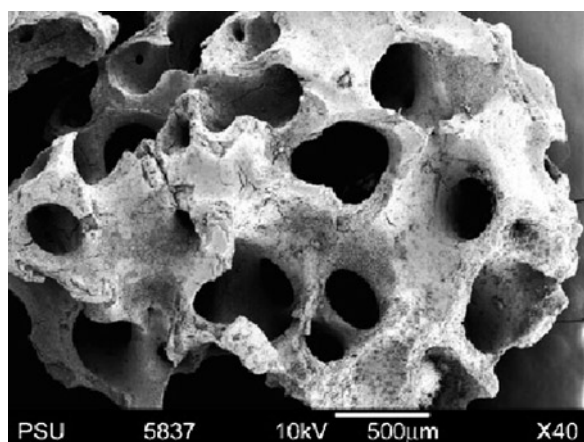


Fig. 2.8 SEM photographs of deproteinized bovine bone (DBB) granules showing the pore structure, size 200–500 µm (original magnification, $\times 400$) (Pripatnanont et al. 2009) (Reprinted with permission from Elsevier)

with the addition of 10% highly purified porcine collagen. As with Bio-Oss, the mineral structure of Bio-Oss Collagen is highly porous, possesses a large internal surface area and functions as a scaffold for bony ingrowth. The collagen component enables convenient handling and simple application but does not function as a barrier. The collagen component allows Bio-Oss Collagen to be easily adapted into the defect. The cohesion of the particles is ensured, even without a membrane. The collagen component is resorbed within 4–6 weeks (<http://www.ostehealth.com>). Bio-Oss Collagen has the capacity to facilitate regeneration of the periodontal attachment apparatus when placed in intrabony defects (Nevins et al. 2005b). No differences in the histological findings were observed following treatment of human intrabony defects with either a bovine-derived xenograft and GTR or a bovine-derived

xenograft mixed with collagen and GTR. Healing was characterized by the formation of new cementum, new periodontal ligament and new bone (Figs. 2.9 and 2.10). Most of the graft particles were surrounded by bone, which in turn points to the highly osteoconductive potential of the graft material (Sculean et al. 2004a).

OsteoGraf/N® (CeraMed Dental, LLC, Lakewood, CO) is a pure, natural form of hydroxylapatite, the major mineral component of tooth enamel and bone. OsteoGraf/N is completely biocompatible and remodels to vital bone at the same rate as host bone.



Fig. 2.9 Healing of human intrabony defects following regenerative periodontal therapy with a bovine-derived xenograft and guided tissue regeneration. Representative histologic view of a healing following treatment with bovine-derived xenograft (BDX) Collagen + guided tissue regeneration (GTR). The healing occurred in formation of new cementum with inserting collagen fibers (C) and new bone (NB) coronally to the notch (N) in the root surface. BDX (G) particles are surrounded by bone. A artifact (Original magnification $\times 50$; van Giesson's connective tissues stain) (Sculean et al. 2004a) (Reprinted with permission from Springer)



Fig. 2.10 Healing of human intrabony defects following regenerative periodontal therapy with a bovine-derived xenograft and guided tissue regeneration. High-power magnification of the regenerated area shown in Fig. 2.9. Bovine-derived xenograft (BDX) particle (G) is surrounded by bone. C new cementum, NPL new periodontal ligament, NB new bone (Original magnification $\times 250$: van Gieson's connective tissues stain) (Sculean et al. 2004a) (Reprinted with permission from Springer)

OsteoGraf/N is the only xenograft that meets all ASTM standards for “Composition of Anorganic Bone for Surgical Implants (F1581-95).” The product is hydrophilic – cohesive consistency when hydrated. It is manufactured as radiopaque, rounded particles and is available in two particle sizes:

- OsteoGraf/N-300 (250–420 μm) packaged in 1-g and 3-g vials
- OsteoGraf/N-700 (420–1,000 μm) packaged in 1-g and 3-g vials

A unique regenerative product is *PepGen P-15*[®] (Dentsply Friadent, Mannheim, Germany), a calcined bovine bone (1,100°C; hydroxyapatite) coated with a pentadecapeptide (P-15, a part of the sequence of collagen). It is available as granulate with a particle size of 0.25–0.42 μm and used in dental applications (Tadic and Eppe 2004). The product is described in more detail in Chap. 5.

2.3.2 Anorganic Porcine-Derived Bone Xenograft

A natural replicate of autologous bone, OsteoBiol[®] Gen-Os (Tecnoss Dental, Turin, Italy) conserves the same intimate structures (matrix and porous form) and presents a high osteoconductive activity (Fig. 2.11). It is biocompatible and bioavailable, as recognized by tests made according to the ISO 10993 method conducted at the Università degli Studi di Torino. Gen-Os is gradually resorbable and provides support in bone neoformation helping to preserve the original graft shape and volume (osteoconductive property). Moreover, thanks to its collagen content, the product facilitates blood clotting and the subsequent invasion of repairing and regenerative cells, favoring restitutio ad integrum of missing bone. Because of its marked “hydrophilia,” it can function as a carrier for selected medication and drugs. Gen-Os must always be hydrated and thoroughly mixed with a few drops of sterile physiological solution to activate its collagen matrix and to enhance its adhesivity; it can also be mixed either with OsteoBiol Gel or with patient’s blood. If necessary it can as well be mixed with the drug selected for surgery. Gen-Os expands up to 50% in volume after hydration with sterile saline: hydrated collagen contained in each granule also increases sensibly biomaterial adhesivity (<http://www.osteobiol.com/products.php>). The particle sizes of the commercialized product are 250–1,000 μm and its porosity 33% (Figueiredo et al. 2010).

The material showed good clinical results when used for augmentation of the alveolar crest and maxillary sinus (Pagliani et al. 2010; Barone et al. 2010), as a filler in postextractive alveolus (Arcuri et al. 2005) and for implant treatment (Fernández et al., 2011;

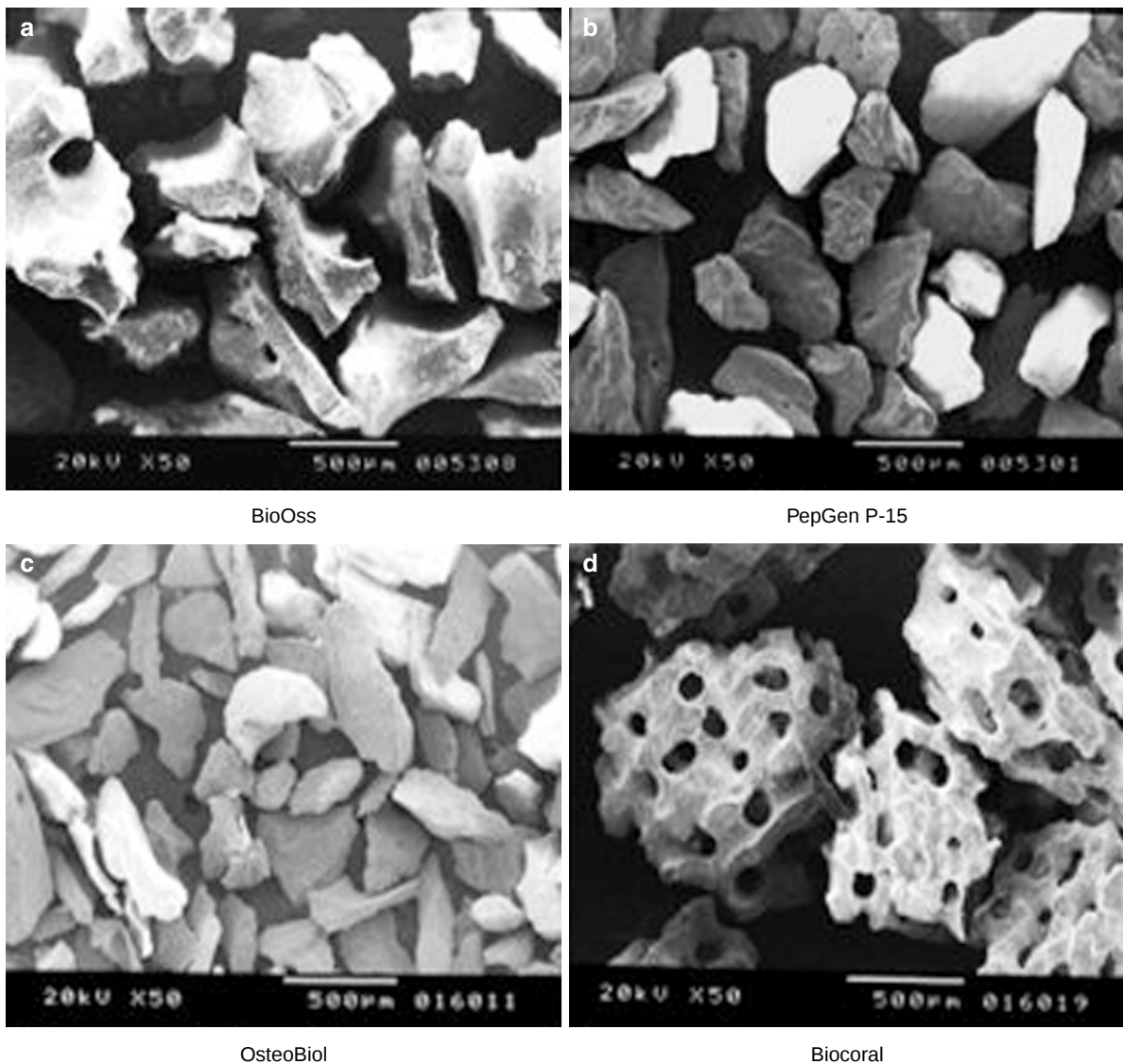


Fig. 2.11 Morphology of the commercial granular samples: (a) Bio-Oss®, (b) PepGen P-15®, (c) OsteoBiol® and (d) Biocoral®. The SEM images show different shapes and structural features

for various particulate materials (Figueiredo et al. 2010) (Reprinted with permission from John Wiley & Sons)

Calvo Guirado et al., 2011). Clinical and histological study suggest that the use of a mixture of collagen gel (OsteoBiol 0, Tecnos) and a collagenated porcine bone (OsteoBiol Gen Os, Tecnos) as a grafting material in combination with a bone cortical lamina (OsteoBiol Lamina Cortical Soft Tecnos) can lead to the

augmentation of the alveolar crest or the maxillary sinus floor prior to or in conjunction with implant placement (Pagliani and Volpe 2010). Histology revealed new bone formation at porcine bone surface, which formed bridges between particles and between particles and preexisting bone. The presence of scalloped

resorption lacunae and new osteons inside the particles indicated ongoing resorption/remodeling of the particles. The histomorphometric analyses showed that the total specimen area consisted of, in average, $56.5 \pm 15.7\%$ of mineralized tissue of which $24.8 \pm 13.9\%$ of the total area was porcine bone particles. Similar data were reported also by Nannmark and Sennerby (2008) who evaluated the response of the bone tissue to prehydrated and collagenated porcine bone, with or without a collagen gel, covered with a collagen membrane (OsteoBiol Evolution, TecnoSS). The histological examinations, performed after 8 weeks, showed an active reabsorption of the materials, by osteoclasts as well as part of remodeling with the formation of osteons within the particles, the presence of mature bone and vascularization of the mineralized part and of the soft tissue, and, finally, the active degradation of the collagen in an animal model. Different collagen/collagenated porcine bone ratios do not influence the bone tissue responses to collagenated porcine bone. Both materials exhibited osteoconductive properties and were starting to be resorbed at 8 weeks (Nannmark and Azarmehr 2010). No studies are presently available for the treatment of periodontal bony defects.

2.3.3 Coralline Calcium Carbonate

Natural coral graft substitutes are derived from the exoskeleton of marine madreporic corals. Researchers first started evaluating corals as potential bone graft substitutes in the early 1970s in animals and in 1979 in humans. The structure of the commonly used coral, *Porites*, is similar to that of cancellous bone, and its initial mechanical properties resemble those of bone. Polyps absorb the calcium ions and carbonic acid present in the seawater to produce aragonite crystals of calcium carbonate, representing 97–99% of the coral exoskeleton. The remaining balance is made up from various elements, such as oligoelements comprising 0.5–1%, magnesium varying from 0.05% to 0.2%, sodium in quantities of 0.4–0.5%, amino acids representing 0.07% and the remainder consisting of traces of potassium (0.02–0.03%), strontium, fluorine and phosphorous in the phosphate form. The oligoelements found in coral are known to play a critical role in the bone mineralization process and in the activation of enzymatic reactions with osteoid cells. Strontium contributes to the mineralization process

and protects calcification. Fluorine, present 1.25–2.5 times more in coral than in bone, helps bone formation through its effects on osteoblast proliferation. The main differences between natural coral and bone include the organic content and the mineral composition. One third of the total weight in bone is composed of organic components, while the coral organic content is limited to 1–1.5%. The mineral composition of bone is mainly hydroxyapatite and amorphous calcium phosphate associated with calcium carbonate, while coral is essentially calcium carbonate (Demers et al. 2002).

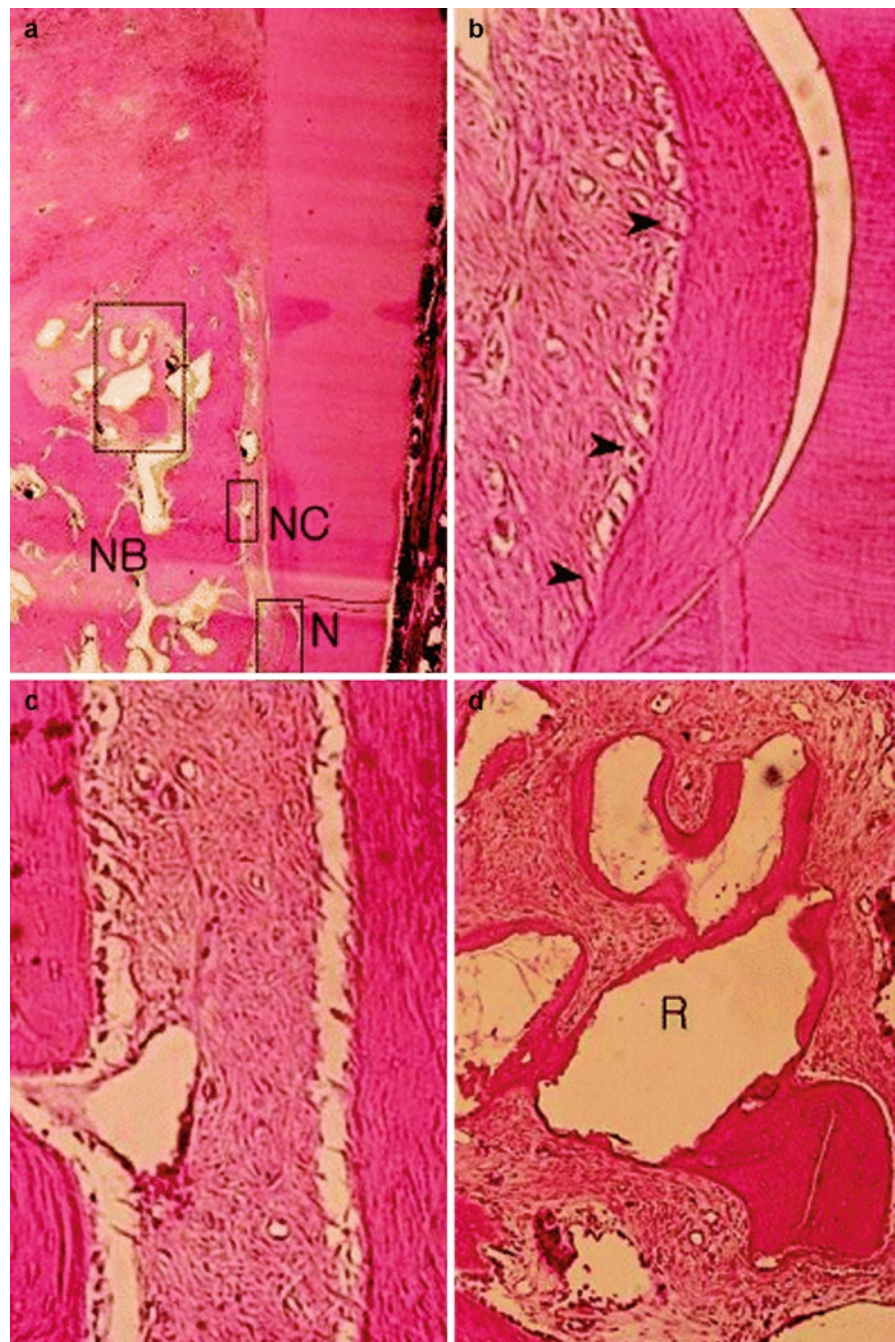
Biocoral (Inotek, Saint Gonnery, France) is a commercially available product.

Biocoral is a resorbable bone graft substitute that belongs to the *Porites* group and had an architecture very similar to that of spongy bone, with a porosity > 45% and interconnecting pores of diameter 100–200 μm (Mora and Ouhayoun 1995; Yukna 1994a). Coral-derived calcium carbonate constructs can be converted to hydroxyapatite by hydrothermal exchange (Ripamonti et al. 2009). The porosity, especially open porosity, of the material seems to influence the speed of colonization and the resorption rate (Piattelli et al. 1997). Quantitative results showed that the larger the porosity volume, the greater was the coral resorption as well as the new bone apposition (Guillemin et al. 1989). The concavities of the matrices biomimeticize the remodeling cycle of the osteonic primate cortico-cancellous bone and promote the ripple-like cascade of the induction of bone formation (Ripamonti et al. 2009).

Biocoral particles *had a very high osteoconductive potential* because no fibrous encapsulation has been reported (Piattelli et al. 1997) (Fig. 2.12). Unlike porous hydroxyapatite, derived from the same coral by heat conversion and made non-resorbable, calcium carbonate is *resorbable*. When implanted into bone tissue, the calcium carbonate crystals gradually resorb to be replaced by mature bone; no fibrous tissue encapsulation, like that reported with hydroxyapatite, was seen (Kim et al. 1996; Piattelli et al. 1997; Gao et al. 1997; Ning et al. 2009). The degradation of Biocoral has been reported to be species specific: 100% degradation in a 3-month period has been reported in a rabbit tibia model (Pollick et al. 1995), while in man most of the particles were present after a 6-month period (Piattelli et al. 1997).

Porous hydroxyapatite (Interpore 200, Irvine, CA) is obtained by the hydrothermal conversion of the calcium carbonate exoskeleton of natural coral into calcium phosphate hydroxyapatite (Saad AlGhamdi et al. 2010b).

Fig. 2.12 (a) Photomicrograph of site implanted with the coral-derived biomaterial showing new bone (NB) and cementum (NC) formation in the notch area (N) (H-E, original magnification $\times 20$). The higher magnifications show the oblique or perpendicular collagen fiber arrangement. (b) A thick layer of new cementum with an oblique or perpendicular collagen fiber arrangement (arrowheads) was observed in the notch area (H-E, original magnification $\times 100$). (c) A well-organized periodontal ligament exhibiting a thinner new cementum layer was observed more coronally (H-E, original magnification $\times 200$). (d) Newly formed osteoid tissue and bone with enclosed osteocytes were deposited around coral-derived biomaterial particles (R) (H-E original magnification $\times 200$) (Kim et al. 2005) (Reprinted with permission from John Wiley & Sons)



Meta-analysis performed by Trombelli et al. (2002) on four selected studies (Kim et al. 1996; Mora and Ouhayoun 1995; Schulz et al. 2000; Yukna 1994a) resulted in a statistically significant difference in CAL gain between coralline calcium carbonate and OFD (weighted mean difference 0.90 mm [95% CI: 0.53–1.27; Q-test for heterogeneity: 6.16 (df = 3), $P = 0.10$]. No significant improvement in probing depth

reduction was observed (weighted mean difference: 0.04 mm).

Reynolds et al. (2003) consistently reported a weighted mean difference in clinical attachment level gain of 0.91 ± 1.94 mm. When changes in bone level were considered, the analysis from three studies (Kim et al. 1996; Mora and Ouhayoun 1995; Yukna 1994a) suggested that coralline calcium carbonate produces a

significant ($P=0.001$) and consistent (heterogeneity not significant) treatment effect (gain in bone fill: 2.21 ± 1.82 mm) (Reynolds et al. 2003). Similar with the previous meta-analysis, no significant improvement in probing depth reduction was noted when treatment with coralline calcium carbonate was compared with OFD (weighted mean difference: 0.09 mm).

Several studies suggested that a bioresorbable calcium carbonate coral implant significantly enhanced space provision for GTR, while alveolar bone formation appeared to be enhanced by its use (Wikesjö et al. 2003; Koo et al. 2005; Polimeni et al. 2004).

2.4 Alloplasts (Alloplastic Synthetic Grafts)

An alloplast is a biocompatible, inorganic synthetic bone grafting material. At present, alloplasts marketed for periodontal regeneration fall into two broad classes: ceramics and polymers. The fate of an alloplastic bone grafting material is dependent primarily on its chemical composition, structure and physical properties (Reynolds et al. 2010).

According to Ashman (1992), an ideal synthetic bone material should be:

1. Biocompatible
2. Able to serve as a framework for new bone formation
3. Resorbable in the long term and have potential for replacement by host bone
4. Osteogenic, or at least facilitate new bone formation
5. Radiopaque
6. Easy to manipulate clinically
7. Not support the growth of oral pathogens
8. Hydrophilic
9. Available in particulate and molded forms
10. Have surface electrical activity (i.e., be charged negatively)
11. Microporous and provide added strength to the regenerating host bone matrix, and permit biological fixation
12. Readily available
13. Nonallergenic
14. Adapt to be effective in a broad range of medical situations (e.g., cancer, trauma and infective bone-destroying diseases)

15. Have a surface that is amenable to grafting
16. Act as matrix or vehicle for other materials (e.g., bone protein inducers, antibiotics and steroids)
17. Have high compressive strength

2.4.1 Polymethylmethacrylate and Polyhydroxyethylmethacrylate (PMMA-PHEMA) Polymers

Polymers present some options that the other groups do not. Like many polymers are potential candidates for bone graft substitutes represent different physical, mechanical, and chemical properties. The polymers used today can be loosely divided into natural polymers and synthetic polymers. These, in turn, can be divided further into degradable and nondegradable types (Nandi et al. 2010).

At present, a biocompatible microporous polymer containing polymethylmethacrylate (PMMA), polyhydroxyethylmethacrylate (PHEMA) and calcium hydroxide is available as a bone grafting material for the treatment of periodontal defects (HTR™ Synthetic Bone – Biopiant, Norwalk, CT). This composite is prepared from a core of PMMA and PHEMA with a coating of calcium hydroxide (Reynolds et al. 2010). It forms calcium carbonate apatite when introduced into the body and interfaces with bleeding marrow (Gross 1997).

The properties of PMMA-PHEMA polymer include a marked hydrophobicity that facilitates hemostasis, extensive microporosity (150–350 μ m interled pore size, which results in a 20–30% material porosity), biocompatibility, an important compressive strength (50,000–60,000 psi) and a negative surface charge (–8 to –10 mV), which is believed to impede development of infection (Ashman 1988). The polymer is hydrophilic and osteophilic, which purportedly aids in stabilization of the healing clot (Reynolds et al. 2010). Its hydrophilicity enhances clotting, and its negative particle surface charge allows adherence to bone. It appears to serve as a scaffold for bone formation when in close contact with alveolar bone (Nasr et al. 1999). PMMA-PHEMA polymer does not produce an inflammatory or immune response after prolonged contact with bone or soft tissue (Ashman and Moss 1977).

Histological evidence of new bone formation on PMMA-PHEMA particles has been reported (Stahl

et al. 1990; Froum 1996; Al Ruhaimi 2001; Haris et al. 1998). Histologic responses varied from gain in closure by epithelial adhesion to new attachment of varying magnitude. At the periphery of some particles, limited bone formation was present. The alveolar bed was remodeling, at times surrounding specific particles (Stahl et al. 1990). Froum (1996) showed that PMMA-PHEMA particles were present and surrounded by connective tissue or bone. PMMA-PHEMA appears to serve as a scaffold for new bone formation when in close contact with alveolar bone.

More clinical studies have provided evidence for the effectiveness of this polymeric grafting material in improving clinical parameters in the treatment of furcations and intrabony defects parameters, relative to open flap debridement (Shahmiri et al. 1992; Yukna 1990, 1994b; Yukna 1994b; Yukna and Yukna 1997; Yukna and Greer 1992; Calongne et al. 2001; Prakash et al. 2010).

In a systematic review (Trombelli et al. 2002), a clinical attachment level gain of 1.9 ± 1.1 mm in the test group, PMMA-PHEMA, and 1.0 ± 0.9 mm in the control group, open flap debridement, was found. The difference between treatments (0.90 mm, SE 0.22) was statistically significant ($P < 0.001$) (Trombelli et al. 2005).

2.4.2 Demineralized Dentin Matrix (DDM)

The organic component of dentin, which accounts for approximately 20% of dentin weight, is mainly type I collagen, a component of bone. Dentin also contains bone morphogenetic proteins (BMPs), which promote the differentiation of mesenchymal stem cells into chondrocytes, and thus enhance bone formation, non-collagen proteins such as osteocalcin and osteonectin, which have been implicated in calcification and dentin-specific proteins including dentin phosphoprotein, also known as phosphophoryn, and dentin sialoprotein (Yagihashi et al. 2009; Kawai and Urist 1989; Feng et al. 1998; Ritchie et al. 1998).

Studies of various mammalian demineralized dentins have shown to be biocompatible, are able to induce differentiation of undifferentiated mesenchymal cells into osteogenic cells, and thus bone and cartilage formation, and are resorbed during the bone remodeling process (Reddi and Huggins 1973; Inoue et al. 1986a, 1986b; Ihoki 1991; Muramatsu et al. 1993; Beertsen et al. 1993; Ymane et al. 1998; Gomes et al. 2001;

Carvalho et al. 2004; Machado et al. 2006; Yagihashi et al. 2009).

Only one study evaluated the effect of implants of allogenic demineralized dentin on bone regeneration and healing in the treatment of infrabony periodontal defects (Movin et al. 1982). The defects were classified as two-wall and combined three- and two-wall bony defects. During healing, no clinical signs of rejection of the dentin implants were observed, but the soft tissue healing was delayed, probably due to a slow resorption of the dentin implants. No conclusive evidence regarding the capacity of allogenic demineralized dentin to induce new connective tissue attachment could be drawn.

2.4.3 Hydroxylapatite (HA)

Synthetic hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, has been available for more than 30 years. It is the primary mineral found in bone. Synthetic hydroxyapatite can be found as porous or nonporous and in ceramic or non-ceramic forms (Kuo et al. 2007).

HAs present remarkable biocompatibility with little inflammatory response when implanted within connective and bone tissues (Froum et al. 1982; Beckham et al. 1971; Jarcho et al. 1977; de Putter et al. 1983). Histologic studies (Froum et al. 1982; Sapkos 1986; Stahl and Froum 1987) demonstrated that healing often occurred with encapsulation of HA graft materials in connective tissue with no indication of new periodontal attachment, osteogenesis or cementogenesis, in the host tissues adjacent to the graft particles. Healing was characterized primarily by formation of a long junctional epithelium. The graft material therefore acted as a biocompatible foreign body within the gingival tissue (Fig. 2.13).

The advantages of using hydroxyapatite are: (1) immunoreaction can be ignored; (2) postoperative morphologic changes and volume decreases do not occur if small blocks and chips are adequately packed during surgery; (3) postoperative adsorption of hydroxyapatite, if any, is slight and slow and is replaced by bone; and (4) cement fixation performed on a layer of hydroxyapatite particles prevents the harmful influence of polyethylene wear particles of cement interface. The clinical disadvantages hydroxyapatite particles are that they tend not to stay in place in a bleeding site, and there is a relatively slow restoration of bone within the assemblage of particles (Oonishi et al. 1997).

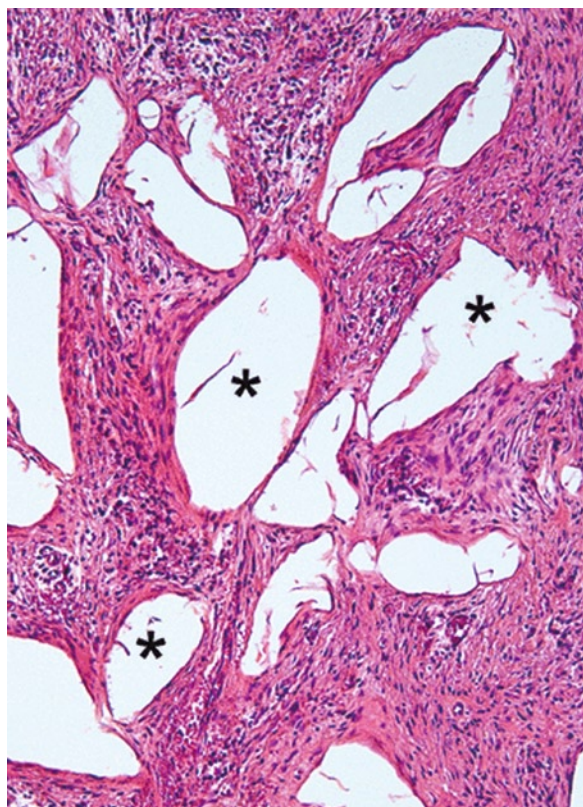


Fig. 2.13 Light micrograph demonstrating fibrous encapsulation of an alloplastic bone-substitute material (asterisks). There is no new bone formation at all (paraffin section stained with hematoxylin and eosin) (Bosshardt and Sculean 2009) (Reprinted with permission from John Wiley & Sons)

Meta-analysis of controlled clinical studies (Galgut et al. 1992; Kenney et al. 1985; Mora and Ouhayoun 1995; Yukna et al. 1998) performed by Trombelli et al. (2002) reported that various forms of HA (porous/non-porous) resulted in significantly greater attachment gain with respect to conventional open flap debridement (OFD) alone (weighted mean difference 1.40 mm, 95% CI: 0.64–2.16). However, meta-analysis revealed that there was highly significant evidence of heterogeneity among studies (Q-test for heterogeneity: 10.72 (df=3), $P=0.01$). Meta-analysis showed also that hydroxyapatite resulted in significantly greater probing depth reduction than the OFD procedure (weighted mean difference 0.98 mm, 95% CI: 0.67–1.29).

The meta-analysis of Reynolds et al. (2003) revealed also the beneficial effect of HA treatment compared with the OFD relative to clinical attachment level gain (1.20 ± 2.22 mm) and probing depth reduction (1.58 ± 1.77 mm), the difference being statistically significant.

Similar positive clinical results were reported when HA grafts were compared to osseous allografts in human vertical lesions. Bowen et al. (1989) reported no significant difference in any of the soft tissue measurements when decalcified freeze-dried bone (DFDBA) and HA were compared. However, both treatment modalities reduced pocket depth and demonstrated a gain in clinical attachment levels. There was 2.2 mm of bone repair with DFDBA and 2.1 mm with HA. These values corresponded to a percent defect fill of 61% for DFDBA and 53% for HA. These values were likewise not statistically different (Bowen et al. 1989). In contrast, Oreamuno et al. (1990) indicated that more clinical resolution of interproximal periodontal defects in humans can be obtained with the use of porous HA than with the use of DFDB.

However, the data and clinical findings suggested that FDBA may have some enhanced reparative potential when compared to granular porous HA in the treatment of periodontal defects in humans. Results showed a mean osseous fill of 2.1 mm for FDBA versus 1.3 mm for granular porous hydroxylapatite ($P=0.07$). A mean clinical attachment gain of 2.2 mm for FDBA versus 1.3 mm for granular porous hydroxylapatite ($P<0.05$), and a mean decrease in probing depths of 3.0 mm for FDBA versus 1.4 mm for granular porous hydroxylapatite ($P<0.5$) was found. FDBA was clinically indistinguishable from host bone, whereas porous hydroxylapatite appeared to be separated from host bone by soft tissue (Barnett et al. 1989).

No significant difference in the use of natural coral skeleton or porous hydroxyapatite for treating 1, 2 wall or combined periodontal bone defects for the clinical parameters was reported (clinical probing depth, clinical attachment, gingival recession, bone fill, % bone fill and crest remodeling). Beneficial effects of using each of the biomaterials were revealed (57.4% for natural coral skeleton, 58.1% for porous hydroxyapatite, $P<0.86$) as opposed to simple debridement (22.2%; $P<0.002$; $P<0.004$) (Mora and Ouhayoun 1995).

There are several available forms of hydroxylapatite:

1. *The polycrystalline ceramic form of pure densely sintered HA* is non-resorbable, osteoconductive, has a low microporosity and act primarily as inert biocompatible fillers (Aichelmann-Reidy and Yukna 1998) (Fig. 2.14). It is prepared in relatively large particle size (18–40 mesh) in most commercially available alloplastic preparations: Calcitec (20–40 Mesh (420–840 μ m) and 40–60 Mesh (250–420 μ m)) (Calcitek,

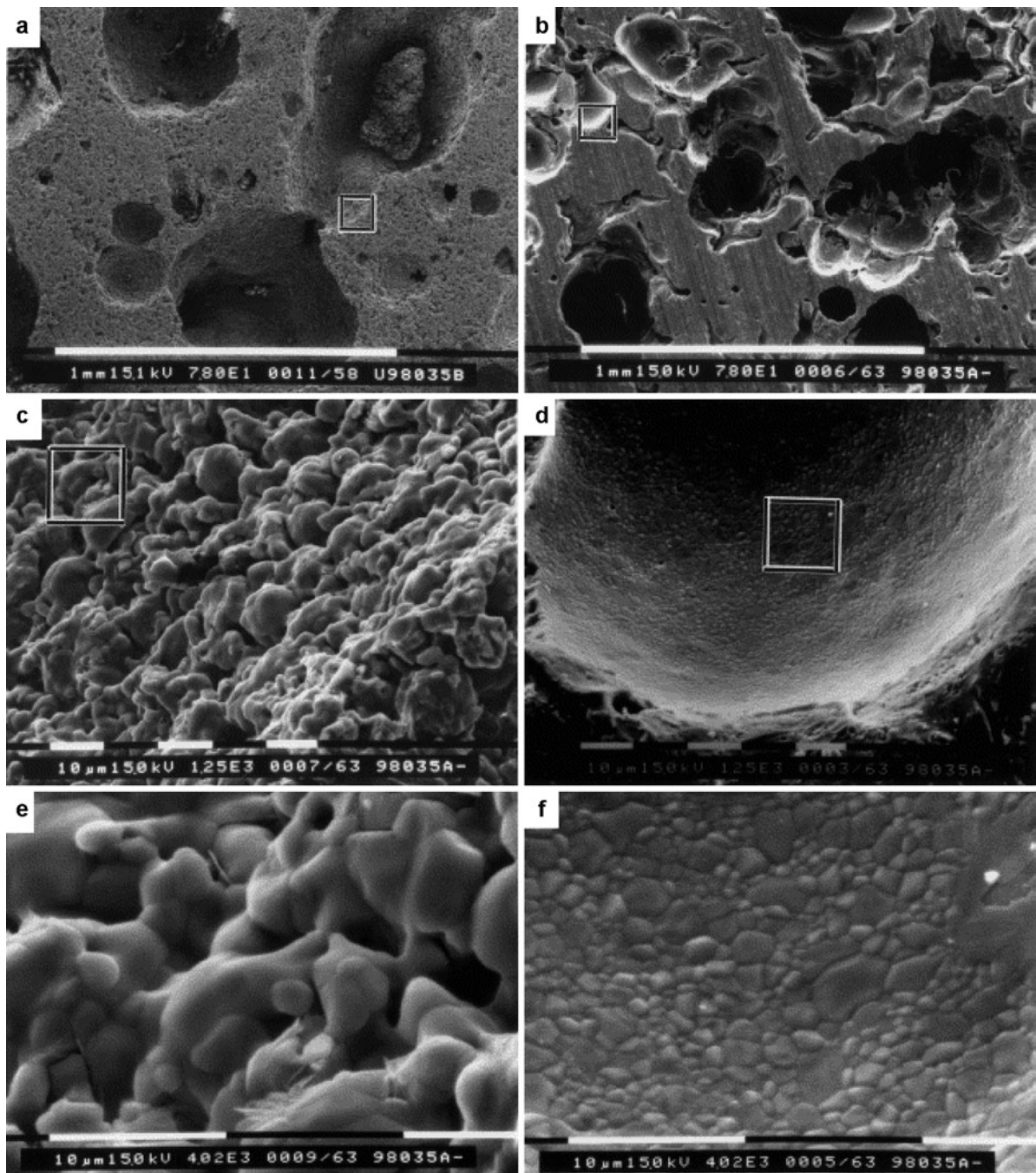


Fig. 2.14 Scanning electronic microscopical observation of an osteoinductive HA ceramic (S-HA) and a non-osteoinductive HA ceramic (J-HA). Note the micropores in osteoinductive HA and the absence of micropores (the dense wall) in non-osteoinductive HA (c) magnification of the square in (a); (e) magnification of the square in (c); (d) magnification of the square in (b); (f) magnification of the square in (d). As described by the producer, S-HA: Hydroxyapatite ceramic rods, 5×6 mm, average

pore size 400 μm , porosity 60–70%, sintered at 1,100°C, prepared by Sichuan Union University (gdu, China); J-HA: Hydroxyapatite ceramic rods, $\phi 3.5 \times 8.5$ mm, provided by Mitsubishi Ceramic Int. (Japan), had a average pore size of 200 μm , a porosity of 70%, and was sintered at 1,200°C (Yuan et al. 1999; Yuan and De Groot 2004) (Reprinted with permission from Elsevier)

Inc., Carlsbad, CA), OsteoGraf/D300 (particle size 250–420 μm) or OsteoGraf/D700 (particle size 420–1,000 μm) (CeraMed Corp., Lakewood, CO).

2. *The coralline porous non-resorbable hydroxylapatite* is a replica of a marine coral skeleton, Porites. After the organic components of the coral have been removed, the aragonite of the coral skeleton is converted to HA by treatment with an ammonium phosphate at elevated temperature and pressure. This hydroxylapatite is formed as small crystals in contrast with the large fused crystals found in the sintered or ceramic-like forms of artificial HA (Kenney et al. 1985).

Human histological studies have revealed ossification in the pores of the hydroxylapatite, along their internal lining with bands of bone varying in width from 20 to 150 μm (Carranza et al. 1987). The ossification of the implant pores and the implant periphery as early as 3 months after implantation became pronounced 12 months after placement (Stahl and Froum 1987). These findings provide evidence that the porous HA has the ability to facilitate osteogenesis within the porous structure of the implant when placed in human periodontal defects (Kenney et al. 1986).

It is marketed in different trade names like Interpore 200 (Interpore International, Irvine, CA) and Pro-Osteon 500R (Interpore Cross International, Irvine, CA, USA).

3. *The resorbable nonceramic hydroxylapatite* is highly microporous, non-sintered (nonceramic), composed of small particles measuring 300–400 μm (35–60 mesh), with a controlled, predictable rate of resorption. As the material resorbs, it acts as a mineral reservoir and predictably induces new bone formation via osteoconductive mechanisms. The material appears to be very biocompatible in both hard and soft tissues (Wagner 1989).

It is marketed in different trade names like Osteogen® (Impladent, NY, USA), OsteoGraf/LD-300 (particles are sized between 250 and 420 μm) (CeraMed Corp., Lakewood, CO) and Cerabone® (Coripharm GmbH & Co. KG, Dieburg, Germany).

4. *Nanocrystalline hydroxyapatite (NHA)*. Researchers have found that nanoparticulate hydroxyapatite not only provides the benefits of traditional hydroxyapatites, but also resorbs (Kuo et al. 2007).

Preliminary experimental studies have shown that nanosized ceramics may represent a promising class of bone graft substitutes due to their improved osseointegrative properties and complete resorption

of the material within 12 weeks, being resorbed by osteoclasts (Thorwarth et al. 2005; Chris Arts et al. 2006; Laschke et al. 2007). NHA exhibited good biocompatibility comparable to that of cancellous bone, as indicated by a lack of venular leukocyte activation after implantation (Laschke et al. 2007) and can promote proliferation and osteogenic differentiation of periodontal ligament cells (Kasaj et al. 2008a; Sun et al. 2007). Biopsy specimens taken at different time intervals from human patients with various types of fractures revealed that the studied nanocrystalline hydroxyapatite paste showed good tissue incorporation and bone regeneration: well-structured cortical and cancellous bone tissue with focal fibrosis of the medullary space. Bone healing and ramifications of trabecular bone could be seen between the implant particles. In all specimens, new bone formation was clearly visible beginning with the deposition of osteoid directly onto the substitute material and secondary mineralization in the presence of cell layers resembling osteoblasts (Huber et al. 2006; Huber et al. 2008). Histological studies of intraosseous periodontal defects treated with NHA revealed, after 7 months, an almost complete resorption of the graft. The healing was characterized by the formation of new connective tissue or long epithelial attachment. New cementum and new bone varied from 0 to 0.86 mm and from 0 to 1.33 mm, respectively (Horvath et al. 2009).

A ready-to-use paste in a syringe, available under the name Ostim™ (Heraeus Kulzer, Hanau, Germany) (NHA), synthetic nanocrystalline hydroxyapatite (NHA) paste containing 65% water and 35% nanostructured apatite particles has been recently introduced in the market. Advantages of this material are the close contact with surrounding tissues, quick resorption characteristics and the large number of molecules on the surface (Schwarz et al. 2006a; Kasaj et al. 2008a) (Fig. 2.15). The needle-shaped HA crystals form agglomerates in transmission electron microscopy (Fig. 2.16). The average crystallite size is 100 nm/20 nm/3 nm, the atomic ratio of calcium phosphorus is 1.67. Ostim paste does not harden after application into the bone and is free of endothermal heating. It is characterized by a large bioactive specific surface area of 106 m²/g. The Ostim syringe in the double-sterile pack can be used to apply paste to the bone defect directly or by means of applicators (Huber et al. 2006).

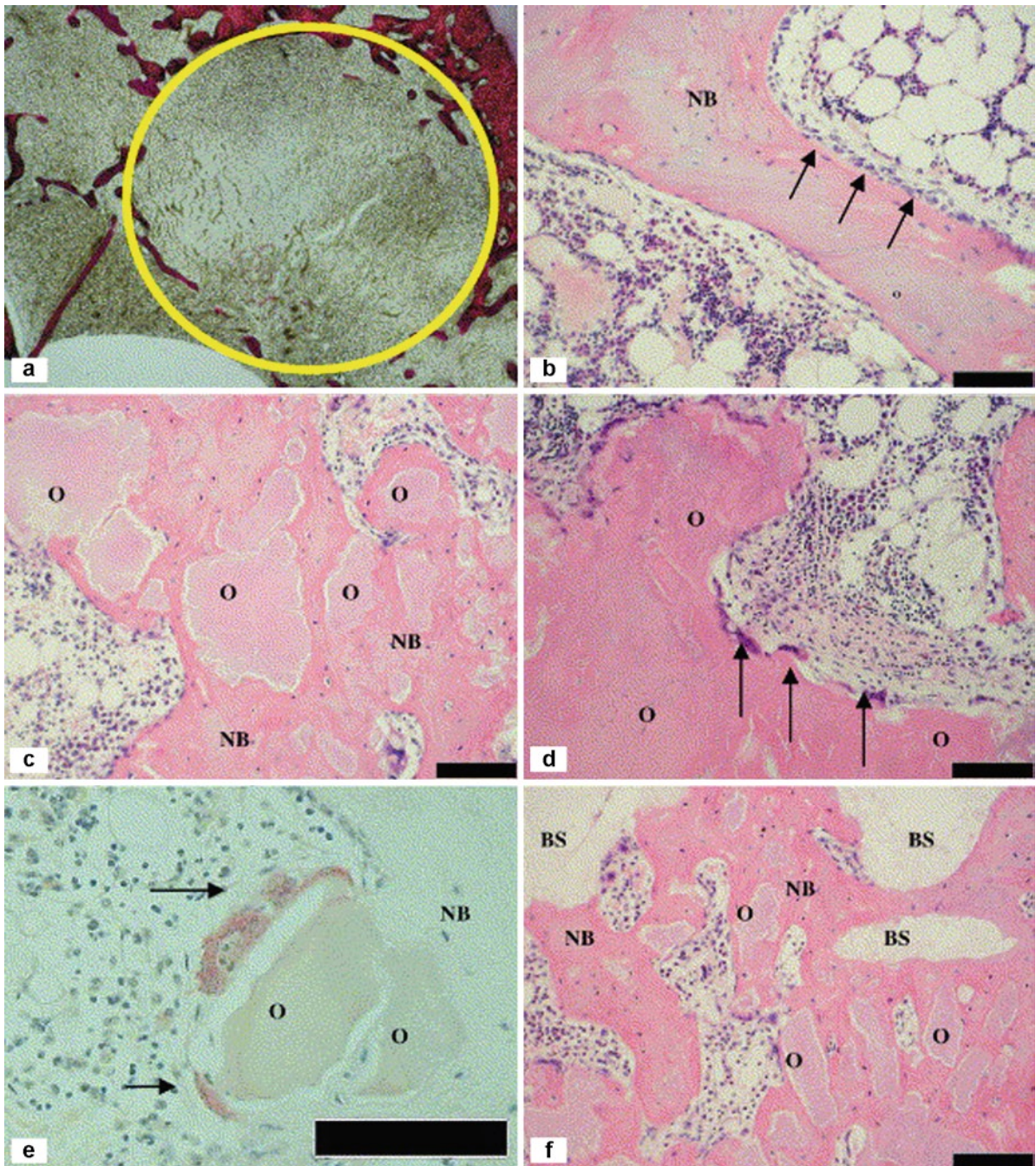


Fig. 2.15 The use of a bioresorbable nanocrystalline hydroxyapatite paste in acetabular bone impaction grafting. Histology results: (a) Section of an empty defect after 8 weeks. The defect borders are denoted by the circle (+ represents 5.5 mm). (b) New bone formation (NB) on a bone graft remnant (BG) with osteoblasts lining the bone graft (arrows) (c) Areas of non-resorbed

Ostim (O) integrated in new bone (NB). (d) Osteoclasts (arrows) against islands of Ostim (O). (e) TRAP staining confirmed the presence of osteoclasts (arrows) on Ostim (O). (f) New bone (NB) osseous-integrated with Ostim (O) and TCP-HA granules (BS). Bar scale B–F 0.1 mm (Chris Arts et al. 2006) (Reprinted with permission from Elsevier)

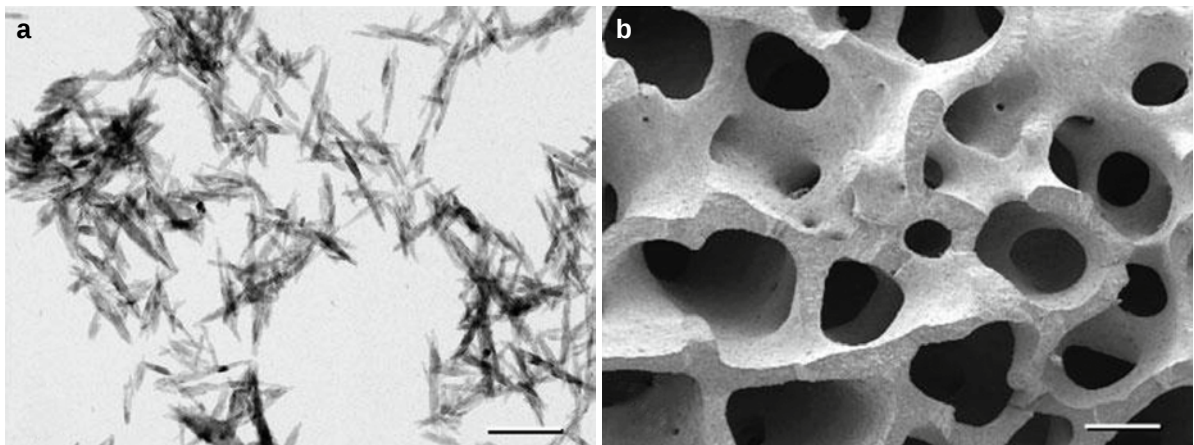


Fig. 2.16 SEM image of Ostim® (a) and REM image of Cerabone® (b). Ostim is a viscous paste, which contains phase pure nanosize hydroxyapatite crystals as a suspension in water (a). In contrast, the granules of Cerabone are synthesized of a

solid hydroxyapatite ceramic (pentacalcium hydroxide triphosphate) with a pore size of $\sim 0.1\text{--}1.5\text{ mm}$ (b). Scale bars: A = 250 μm ; B = 350 μm (Laschke et al. 2007) (Reprinted with permission from John Wiley & Sons)

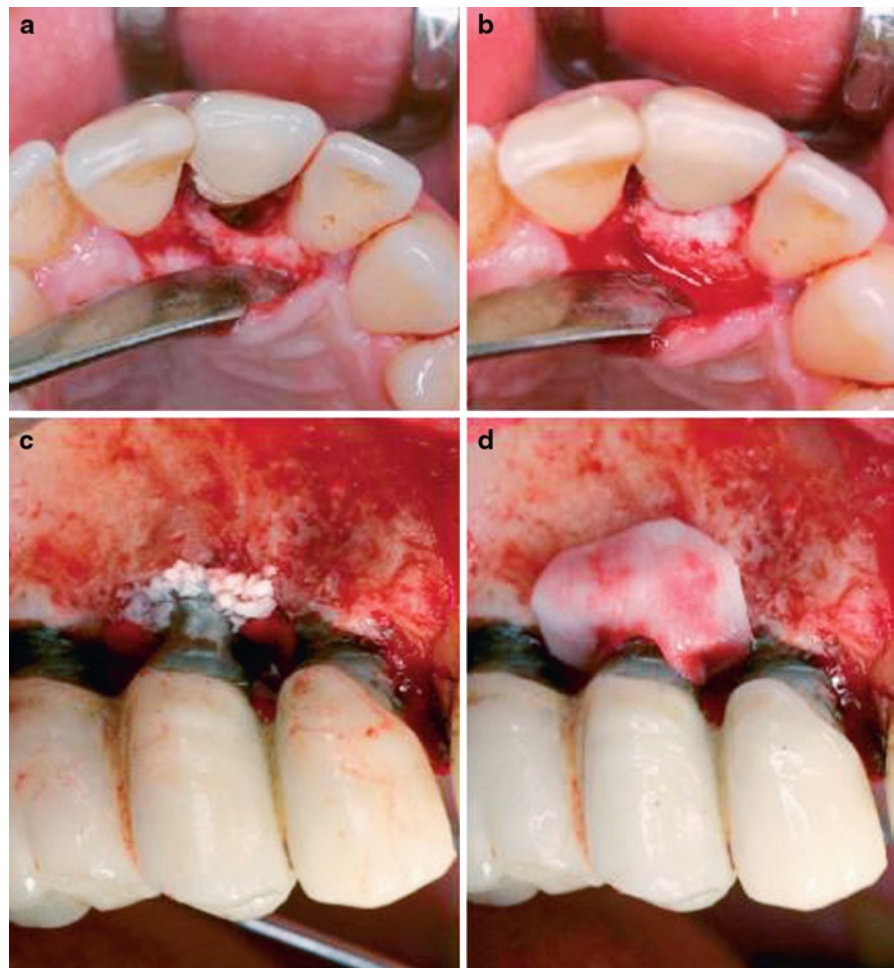


Fig. 2.17 (a) Semi-circumferential intrabony defect. (b) Situation following application of nanocrystalline hydroxyapatite. (c) Situation following application of bovine-derived xenograft. Bio-Gide collagen membrane was trimmed and adapted over the oral and (d) the vestibular aspect of the defect (Schwarz et al. 2006a) (Reprinted with permission from John Wiley & Sons)



Fig. 2.18 Surgical treatment of intrabony periodontal defects with a fully synthetic nanocrystalline hydroxyapatite (nano-HA) paste (Ostim®; Heraeus Kulzer, Hanau, Germany) containing 65% water and 35% of nanostructured apatite. (a) The intraoperative clinical image shows a deep intrabony defect distal of the

lower right first premolar. (b) Nano-HA paste placed. (c) Clinical situation 6-month postoperative. (d) Presurgical radiograph. (e) 6-month postoperative radiograph (Heinz et al. 2010) (Reprinted with permission from Springer)

The NHA has been proven to be useful for augmentation procedures in osseous defects (Moghadam et al. 2004; Thorwarth et al. 2005) (Fig. 2.17), in the peri-implantitis lesions (Schwarz et al. 2006a; Schwarz et al. 2008; Schwarz et al. 2009) and intraosseous periodontal defects (Fig. 2.18) (Kasaj et al. 2008a; Horvath et al. 2009; Heinz et al. 2010). After 6 months, the treatment of intrabony periodontal defects with an NHA paste led to significantly improved clinical outcomes when compared with open flap debridement alone. Kasaj et al. (2008a) reported a reduction in mean PD from 7.4 ± 1.3 mm to 3.4 ± 1.2 mm and a change in mean CAL from 8.0 ± 1.3 mm to 4.4 ± 1.7 mm for the test group, whereas in the control group the mean PD decreased

from 7.4 ± 0.8 mm to 4.9 ± 0.9 mm, and mean CAL decreased from 8.1 ± 1.2 mm to 6.4 ± 1.3 mm. Similar results were reported by Heinz et al. (2010) who showed that the sites treated with NHA paste showed a reduction in mean PPD from 8.3 ± 1.2 to 4.0 ± 1.1 mm and a gain in PBL of 4.3 ± 1.4 mm, whereas in the control group, the mean PPD changed from 7.9 ± 1.2 mm to 5.0 ± 1.2 mm and PBL gain was 2.6 ± 1.4 mm. Horvath et al. (2009) reported a PD reduction of 4.0 ± 0.9 mm and a CAL gain of 2.5 ± 0.8 mm, on average after the NHA treatment.

5. *Fluorohydroxyapatitic (FHA) biomaterial.* The natural architecture of some calcified algae offers a surface that is similar to that of bone (Kasperk et al. 1988) Damien and Revell 2004.

The commercially available porous biomaterial FRIOS® Algipore® (Friadent GmbH, Mannheim, Germany) is manufactured from calcifying marine algae (*Corallina officinalis*). The particles contain a pore system with a mean diameter of 10 μm that is periodically septated (mean interval 30 μm) and interconnectively microperforated (mean diameter of perforations 1 μm). Every pore is limited by one layer of small FHA crystallites with a size of 25–35 nm (Fig. 2.19). The impression of a bilayer texture of the walls arises by the contact of the layers of adjacent pores (Schopper et al. 2003). This material is biocompatible, osteoconductive and has an additional desirable property of being slowly resorbable and replaced by newly formed bone (Ricci et al. 1992; Schopper et al. 2003; Ewers et al. 2004; Klongnoi et al. 2006). Clinical investigations demonstrated good results in sinus augmentation (Ricci et al. 1992; Schopper et al. 2003; Klongnoi et al. 2006; Lee et al. 2009). In an animal model, they suggest that C-Graft particles provide a scaffold for the regeneration of new bone and cementum in periodontal bone defects in dog (Nakanishi et al. 2009).

Biostite® (Vebas s.r.l., S. Giuliano Milanese, Italy) is a mixture of synthetic HA (88.0%, granulometry of 160–200 μm , total porosity of 60%), equine type I collagen (9.5%) and chondroitin sulfate (2.5%). Chemically, Biostites particles demonstrated a major phase represented by polycrystalline synthetic HA ($\geq 99\%$), little presence of β -tricalcium phosphate and CaO ($\leq 1\%$) and a Ca–P ratio ranging from 1.665 to 1.697 (Scabbia and Trombelli 2004). The material has proved to be highly biocompatible and osteoconductive (Benqué et al. 1985; Serre et al. 1993), as well as actively reabsorbable (Parodi et al. 1996). It has been shown that Biostite®, may directly affect osteoblasts by enhancing chondro/osteogenic gene expression and cytoskeleton-related signaling pathways, which may contribute to its clinical efficacy (Sibilla et al. 2006). Clinical studies have shown favorable effects of Biostite® in improving attachment and probing depth when used for the treatment of intraosseous defects, similar with those obtained with an anorganic xenograft (Bio-Oss®, Geistlich Pharma AG, Wolhusen, Switzerland) (Scabbia and Trombelli 2004).

Biphasic alloplastic materials produced by sintering hydroxyapatite (HA) and tricalcium

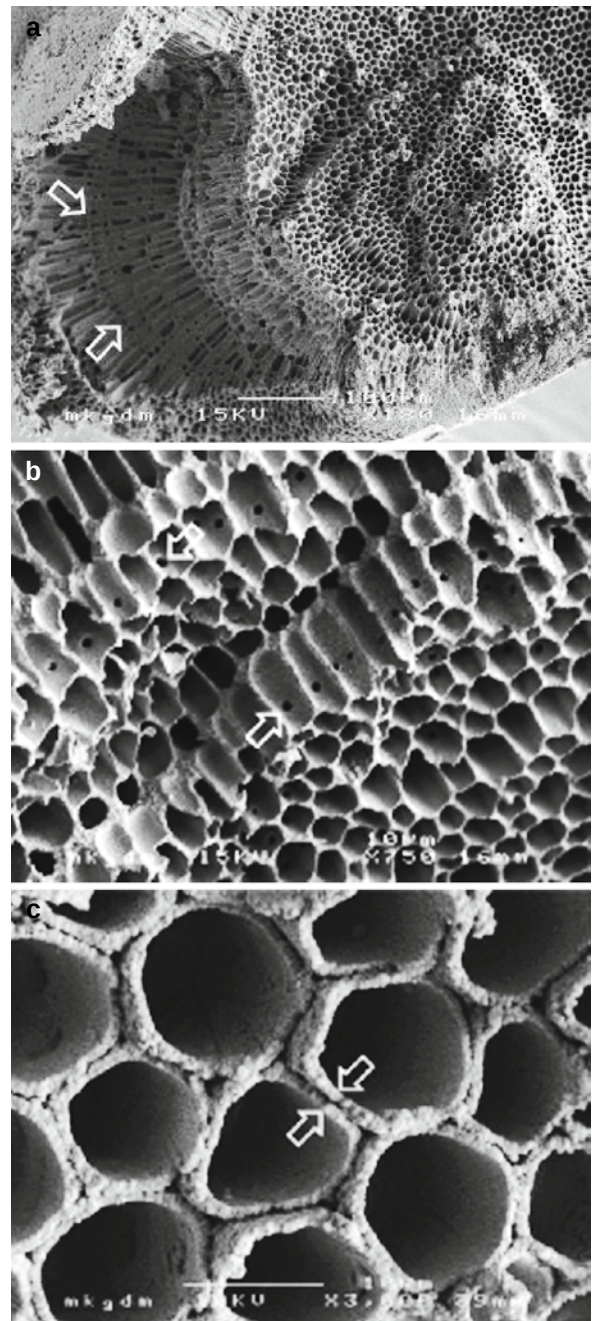


Fig. 2.19 (a) Scanning electronmicroscopic examination visualizes the spatial arrangement of the pore system within the fluorohydroxyapatite (FHA) FRIOS Algipore biomaterial particles. Arrows show the periodical septation of the pores. (b) Microperforations (arrows) within the fragile walls of the pores connect adjacent pores with each other. (c) Small FHA crystallites are assembled within the walls of the pores. Every pore is limited by one FHA layer. The impression of a bilayer texture of the walls (arrows) arises by the contact of the layers of adjacent pores. (a) Bar = 100 μm . (b, c) Bar = 10 μm (Schopper et al. 2003) (Reprinted with permission from John Wiley & Sons)

phosphate include Calcitec® Inc. (Austin, TX), Osteogen® (Impladent Ltd, Holliswood, NY), Tricos® (Baxter, Bern, Switzerland), MBCP (Biomatlante, Vigneux de Bretagne, France), Ceraform® (Teknimed SA, Vic-en Bigorre, France) and Bone Ceramic® (Straumann, Basel, Switzerland).

2.4.4 Calcium Phosphate Cement (CPC)

Among the materials used for bone and tissue regeneration, calcium phosphate cements are gaining special interest due to their biomimetic nature and potential use as controlled release systems (Table 2.4). These cements are prepared by mixing a liquid phase with a solid phase to create a workable paste that sets into a solid material (Ambard and Mueninghoff 2006; Tamimi et al. 2008; Burguera et al. 2008). The injectable CPC required minimal manipulation to fill and mold the defect wall compared to the placement of a membrane with the GTR procedure (Shirakata et al. 2007). Unfortunately, CPCs have been reported to suffer from some problems, such as prolonged setting time and the inability to set in the presence of blood. Recently, new improved cements have been developed, especially suited for the filling of peri-implant and periodontal defects. They are reported to have a short setting time (around 10 min), fast biodegradation and the possibility to be applied with a syringe (Comuzzi et al. 2002). Premixed calcium phosphate cements were also developed (Xu et al. 2007).

The histological and histomorphometrical examinations confirmed the excellent bone biocompatibility

and osteoconductive properties of the used CaP cement. The material did not evoke any inflammatory response, but favored new bone formation comparable with autologous bone grafting (Aral et al. 2008; Yuan et al. 2000) (Fig. 2.20). This material had been used as a bioabsorbable barrier for guided tissue regeneration in periodontal defects (AlGhamdi et al. 2010b). Animal models revealed that CPC have a high biocompatibility and has an ability to act as a stable scaffold for bone formation and provide adequate space for periodontal tissue regeneration (Fujikawa et al. 1995; Shirakata et al. 2002; Hayashi et al. 2006; Shirakata et al. 2007; Sugawara et al. 2008; Lee et al. 2010). Moreover, in vivo, it cures into an osteoconductive carbonated apatite with chemical and physical characteristics similar to the mineral phase of bone, which subsequently is replaced by natural bone (Shirakata et al. 2008; Constantz et al. 1995; Cohen and Whitman 1997; Elder et al. 2000). Resorption of calcium phosphate ceramics occurs by dissolution or is cell mediated, for example, by foreign body giant cells and osteoclasts (Aral et al. 2008; Yuan et al. 2000). Fast resorption of residual material is desirable to avoid the risk for infection and increase the amount of regenerated periodontal tissue. In line with this, histologic results indicated that when holes were drilled into the hardened CPC, resorption of the CPC mass improved, supposedly by increasing the surface area of the material and allowing increased vascular supply (Shirakata et al. 2002). Experimental studies have proposed the CPCs as a suitable graft for repairing root perforations (Noetzel et al. 2006), sinus augmentation (Aral et al. 2008), as a filler for bone defects around oral implants (Comuzzi et al. 2002) and in alveolar ridge augmentation (Sato et al. 2009).

Table 2.4 Main calcium phosphate compounds

Ca/P molar ratio	Compound	Formula	Symbol
0.5	Monocalcium phosphate monohydrate	$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$	MCPM
0.5	Monocalcium phosphate anhydrous	$\text{Ca}(\text{H}_2\text{PO}_4)_2$	MCPA
1.0	Dicalcium phosphate dihydrate	$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	DCPD
1.0	Dicalcium phosphate anhydrous	CaHPO_4	DCPA
1.33	Octacalcium phosphate	$\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}$	OCP
1.5	α -Tricalcium phosphate	$\alpha\text{-Ca}_3(\text{PO}_4)_2$	α -TCP
1.5	β -Tricalcium phosphate	$\beta\text{-Ca}_3(\text{PO}_4)_2$	β -TCP
1.67	Hydroxyapatite	$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	HA
2.0	Tetracalcium phosphate	$\text{Ca}_4(\text{PO}_4)_2\text{O}$	TTCP or TetCP

Source: Kamitakahara et al. (2008). Reprinted with permission from Sage Publications

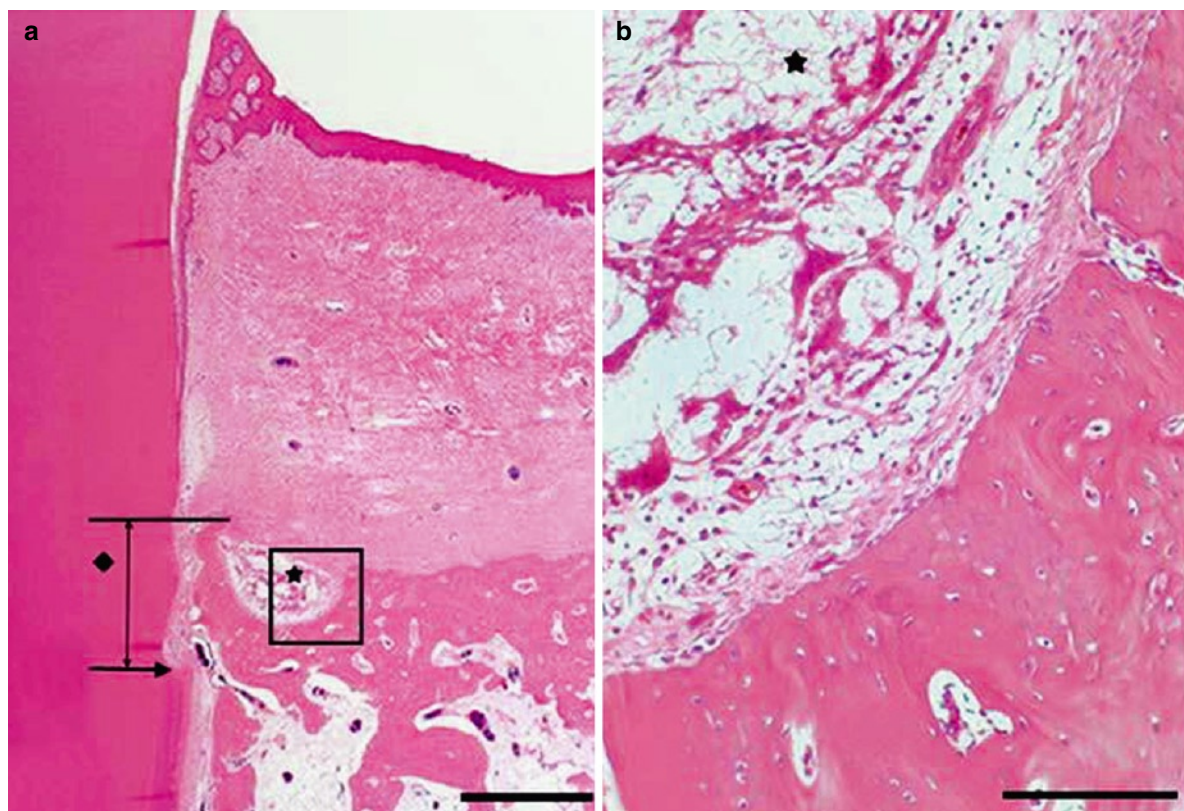


Fig. 2.20 Osteoconductive effects of calcium phosphate glass cement grafts in rabbit calvarial defects. Surgical sections from the calcium phosphate glass cement (CPGC) group. **(a)** Histologic view of the CPGC group. Most particles were resorbed and new bone was formed above the notch (H–E, $\times 20$; base of reference notch [bN]: arrow; height of new bone: black diamond; CPGC

particle: black star; bar = 2 mm). **(b)** Histologic view of magnified black square area ($\times 200$). Osteoblast-like cells were observed around remaining particles. Peripheral new bone was woven bone with isolated osteocytes (H–E, $\times 200$; CPGC particle: black star; NB: new bone; WB: woven bone; bar = 0.1 mm) (Lim et al. 2010) (Reprinted with permission from John Wiley & Sons)

When CPC was used in human studies for periodontal bone repair, tooth mobility resulted in early fracture and eventual exfoliation of the rigid and brittle implants (Brown et al. 1998; Xu et al. 2002). Controversial results were obtained from clinical studies, CPCs being either significantly better than hydroxyapatite ceramic granules (Rajesh et al. 2009) either failing to demonstrate any superior clinical outcomes for the CPC compared to the OFD when used in treating intraosseous periodontal defects (Shirakata et al. 2008). Recently, Mellonig et al. (2010) evaluated the clinical and histologic results of a calcium phosphate bone cement in the treatment of human periodontal intraosseous defects. At 6 months, results demonstrated that all defects resulted in probing depth reduction and in clinical attachment level

gain. However, no site showed periodontal regeneration and there was no new bone formation. New cementum and connective tissue were limited to 0.2 mm or less. Large deposits of the bone cement were noted encapsulated in connective tissue (Mellonig et al. 2010).

In recent years, lots of phosphate cements have been developed and studied (Table 2.5). Common components besides tetracalcium phosphate, dicalcium phosphate dihydrate or anhydrous are monocalcium phosphate monohydrate and anhydrous, octacalcium phosphate, tricalcium phosphate, hydroxyapatite and fluorapatite, with different additives like carbonates, sulfates or metallic oxides. Water, calcium- or phosphate-containing solutions, organic acids or aqueous solutions of polymers are

Table 2.5 List of commercial calcium phosphate cements with their composition (when available)

Company	Cement name	Components	End-product
ETEX	α -BSM	Powder: ACP (50%), DCPD (50%)	Apatite
	Embarc	Solution: H ₂ O (unbuffered saline solution)	
	Biobon		
Stryker-Leibinger Corp.	BoneSource	Powder: TetCP (73%), DCP (27%)	Apatite
		Solution: H ₂ O, mixture of Na ₂ HPO ₄ and NaH ₂ PO ₄	
Teknimed	Cementek®	Powder: α -TCP, TetCP, Na Glycerophosphate	Apatite
		Solution: H ₂ O, Ca(OH) ₂ , H ₃ PO ₄	
	Cementek® LV	Powder: α -TCP, TetCP, Na Glycerophosphate, dimethylsiloxane Solution: H ₂ O, Ca(OH) ₂ , H ₃ PO ₄	Apatite
Biomet	Calcibon® (previously called "Biocement D")	Powder: α -TCP (61%), DCP (26%), CaCO ₃ (10%), PHA (3%) Solution: H ₂ O, Na ₂ HPO ₄	Apatite
	Mimix™	Powder: TetCP, α -TCP, C ₆ H ₅ O ₇ Na ₃ · 2H ₂ O Solution: H ₂ O, C ₆ H ₈ O ₇	Apatite
	QuickSet Mimix™	Powder: nf ^a Solution: nf ^a	Apatite
Mitsubishi Materials	BiopeX®	Powder: α -TCP (75%), TetCP (20–18%), DCPD (5%), HA (0–2%) Solution: H ₂ O, sodium succinate (12–13%), sodium chondroitinsulphate (5–5.4%)	Apatite
	BiopeX®-R	Powder: α -TCP, TetCP, DCPD, HA, Mg ₃ (PO ₄) ₂ , NaHSO ₃ Solution: H ₂ O, sodium succinate, sodium chondroitin sulphate	
Kyphon	KyphOs™	Powder: α -TCP (77%), Mg ₃ (PO ₄) ₂ (14%), MgHPO ₄ (4.8%), SrCO ₃ (3.6%) Solution: H ₂ O, (NH ₄) ₂ HPO ₄ (3.5 M)	Apatite
Skeletal Kinetics	Callos™	Powder: nf ^a Solution: nf ^a	Apatite
Shanghai Rebone Biomaterials Co, Ltd	Rebone	Powder: TetCP, DCP Solution: H ₂ O ^b	Apatite
Synthes-Norian	Norian® SRS	Powder: α -TCP (85%), CaCO ₃ (12%) MCPM (3%)	Apatite
	Norian® CRS	Solution: H ₂ O, Na ₂ HPO ₄ ^c	Apatite
	Norian® SRS Fast Set Putty	Powder: nf ^a	
	Norian® CRS Fast Set Putty	Solution: nf ^a	
	chronOS™ chronOS™ Inject	Inject Powder: β -TCP (73%), MCPM (21%), MgHPO ₄ · 3H ₂ O (5%), MgSO ₄ (0.1%), Na ₂ H ₂ P ₂ O ₇ (<1%) Solution: H ₂ O, sodium hyaluronate (0.5%)	Brushite

(continued)

Table 2.5 (continued)

Company	Cement name	Components	End-product
Kasios	Eurobone®	Powder: β -TCP (98%), $\text{Na}_4\text{P}_2\text{O}_7$ (2%) Solution: H_2O , H_3PO_4 (3.0 M), H_2SO_4 (0.1 M)	Brushite
CalciphOs	VitalOs	Component 1: β -TCP (1.34 g), $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$ (0.025 g), H_2O , salts (0.05 M pH 7.4 PBS solution) Component 2: MCPM (0.78 g), $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (0.39 g), H_2O , H_3PO_4 (0.05 M)	Brushite

Source: Bohner et al. (2005) (and references therein). Reprinted with permission from Elsevier

The end product of the reaction can be either an apatite (calcium deficient, carbonated, etc.) or brushite

^aNot found in the literature or on the web

^bAssumed composition based on the scientific literature

^cEstimated composition

^dThe cement consists of two liquids in which the various powder components are dispersed

used as cement liquids. The primary role of the liquid is to provide a vehicle for the dissolution of the reactants and precipitation of the products, although it may sometimes contain a reactant for the cement setting reactions (Noetzel et al. 2006). A nonrigid and high-strength CPC by incorporating tetracalcium phosphate and chitosan, a natural elastomeric and biocompatible biopolymer, into the conventional CPC was developed (Xu et al. 2002; Xu et al. 2006). The powder of Augmentech AT (Wetzlar, Germany) consists of tricalcium phosphate (TCP), magnesium phosphate, magnesium hydrogen phosphate and strontium carbonate. The liquid is a watery solution of diammonium hydrogen phosphate (Noetzel et al. 2006). Norian® PDC™ (Shofu Inc., Kyoto, Japan) is injectable, moldable, fast setting, bioabsorbable and has high compressive strength. It is composed by a powder mix composed of α -tricalcium phosphate (a $\text{Ca}_3[\text{PO}_4]_2$), monocalcium phosphate monohydrate ($\text{Ca}[\text{H}_2\text{PO}_4]_2 \cdot \text{H}_2\text{O}$) and calcium carbonate (CaCO_3) mixed with a solution of sodium phosphate. It began to harden at a physiologic temperature and pH, and the final compressive strength was 55 MPa (compared to 1.9 MPa for cancellous bone). Its pore diameter was 300 Å. The powder and liquid are added separately in a sterile capsule and need to be blended inside the capsule using an amalgam mixer-like apparatus for 20 s. The mixed CPC should be injected into the defects within 5 min (Shirakata et al. 2008). Norian PDC is bioabsorbable and is provided as a capsule composed of powder and liquid. Once the paste sets, it is moldable and injectable within 5 min. Hardened Norian PDC has enough

compressive mechanical strength to be kept in the defect by itself without the use of a membrane. After solidification, the CPC can act as an occlusive space maintainer, providing adequate space for bone regeneration (Sato et al. 2009).

The fabrication of CPC is a versatile process which yields a variety of tailor-made injectable pastes and sets cement materials with different physicochemical and mechanical properties. The ultimate properties of the cement will depend on the characteristics of the solid and aqueous phase and the reaction conditions. One feature of special interest in cements is the fact that they are intrinsically porous. They have an important percentage of porosity within the nano/submicron size range. While porosity can be a limitation for the use of these materials in high-load-bearing applications, for example, vertebroplasty, it is vital for other applications. Porosity is sought to enhance a material's resorbability and the extent of bioactivity by increasing the surface area available for reaction. In the same way, their inherent porosity makes these materials good carriers for controlled drug delivery systems (Espanol et al. 2009; Ginebra et al. 2006a, b).

The possibility to use CPCs not only as bone substitutes but also as carriers for local and controlled supply of drugs is very attractive and can be useful in treatments of different skeletal diseases, such as bone tumors, osteoporosis or osteomyelitis, which normally require long and painful therapies. Unlike calcium phosphate ceramics employed as drug delivery systems, where the drugs are usually absorbed on

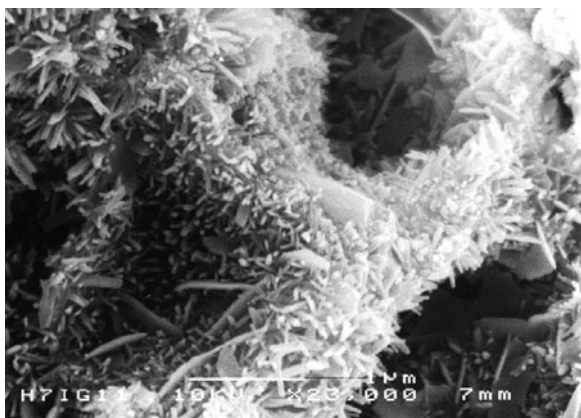


Fig. 2.21 Microstructure of an apatitic calcium phosphate cement after setting, showing the micro-/nanoporous structure formed by the entanglement of the precipitated crystals (Ginebra et al. 2006a) (Reprinted with permission from Elsevier)

the surface, in CPCs the drugs can be incorporated throughout the whole material volume, by adding them into one of the two cement phases (Fig. 2.21). This fact can facilitate the release of drugs for more prolonged times (Ginebra et al. 2006a).

The studies about incorporation of drugs into CPC cover different aspects. In the first place, it is necessary to verify that the addition of the drug (either to the liquid or the solid phases of the cement) does not interfere in the setting reaction, modifying the physicochemical properties, not only in terms of the setting and hardening mechanisms but also with respect to the rheological behavior. Second, it is necessary to characterize the kinetics of drug release in vitro. Subsequently, the effectiveness of the cement to act as carrier for drug delivery in vivo must be assessed. And finally, the clinical performance of the drug delivery system must be evaluated (Ginebra et al. 2006a).

CPCs have been proposed as carriers for biologically active peptides, such as antibiotics (Yu et al. 1992; Bohnert et al. 1997; Blom et al. 2001) and bone growth factors (Otsuka et al. 1994; Meraw et al. 2000; Wikesjö et al. 2002; Sorensen et al. 2004). A recent large-scale, prospective, blinded, and randomized controlled clinical trial study demonstrated that the use of rhPDGF-BB + β -TCP was safe and effective in the treatment of periodontal osseous defects (Nevins et al. 2005a). The incorporation of platelet-derived growth factor-BB (PDGF-BB) with β -tricalcium phosphate was approved by the FDA in 2004. GEM-21 S™ is a

completely synthetic grafting system for bone and periodontal regeneration launched in 2005. This system is composed of a purified platelet-derived growth factor-BB (PDGF-BB) and β -tricalcium phosphate matrix (AlGhamdi et al. 2010b).

2.4.5 β -Tricalcium Phosphate (TCP)

Tricalcium phosphate is a porous calcium phosphate compounds (Yamada et al. 2010). Alpha and beta tricalcium phosphate are produced similarly, although they display different resorption properties. The crystal structure of alpha tricalcium phosphate (α - $\text{Ca}_3(\text{PO}_4)_2$) is monoclinic and consists of columns of cations, while the beta tricalcium phosphate has a rhombohedral structure. The former is formed by heating the latter above 1,180°C and quenching in air to retain its structure. Alpha form is less stable than beta and forms the stiffer material calcium-deficient hydroxyapatite when mixed with water (Sukumar and Drízhál 2008; TenHuisen and Brown 1998).

β -Tricalcium phosphate (β -TCP) is a porous form of calcium phosphate, with similar proportions of calcium and phosphate to cancellous bone (Reynolds et al. 2010). However, the compressive strength of porous TCP reaches only 1/20 of cortical bone (Gao et al. 1997; Jarcho 1981). Numerous studies have shown that calcium TCP support the attachment, proliferation and differentiation of osteoblasts and mesenchymal cells as well as *bone growth* (von Arx et al. 2001; Aybar et al. 2004; Haimi et al. 2009; Jang et al. 2008; Kamitakahara et al. 2008). Tricalcium phosphate ceramic is *biocompatible* (Metsger et al. 1982) and *osteoconductive* (Knabe et al. 2000; Ignatius et al. 2001; Hashimoto-Uoshima et al. 1995). The exact mechanism(s) by which β -TCP exerts osteoconductivity were documented only recently. It was showed that primary human osteoblasts (HOBs) seeded into the β -TCP scaffolds expressed significantly higher levels of osteogenic genes, compared to those cultured on tissue culture plastic; meanwhile, these cells showed sevenfold increase in $\alpha 2$ integrin subunit gene expression and the activation of the mitogen-activated protein kinase (MAPK)/extracellular-related kinase (ERK) signaling pathway. In addition, the osteogenic conduction by β -TCP scaffolds was attenuated directly by

inhibiting MAPK/ERK or indirectly by blocking the $\alpha 2\beta 1$ integrin signaling pathway. It seems that β -TCP scaffold exerts osteoconductivity through $\alpha 2\beta 1$ integrin and downstream MAPK/ERK signaling pathway (Lu and Zreiqat 2010a, 2010b).

Physicochemically, β -TCP is a resorbable material with $\geq 99\%$ phase purity (Tadic and Epple 2004), total microporosity and a homogeneous ceramic sinter structure. Thus, optimal matrix for the formation of new bone is available immediately after implantation. Bioresorbability of the calcium phosphate ceramics is governed not only by the solubility of the constituents of the material, but also by morphology implying porosity and pore structure (Kamitakahara et al. 2008). The intergranular spaces provide a scaffold for ingrowths of blood vessels for nutrition of the newly formed bony structures. From the initial stage of bone regeneration, the material is resorbed (Fig. 2.22). Its slow biodegradation property, within 24 months the material is completely metabolized, harmonizes with bone formation and remodeling process and results in a displacement of the material to bone (Ellinger et al. 1986; Artzi et al. 2004; Bokan et al. 2006; Kamitakahara et al. 2008). The resorption mechanism of the formal β -TCP is controversial. It was suggested that the mechanism is mainly dissolution in biological liquids because of the absence of osteoclasts around the materials in rabbit's experiments (Lu et al. 1998). Cell-mediated bioresorption was also proposed to be a predominant factor in the process of biodegradation of

β -TCP in dog experiments, as considerable numbers of osteoclast-like giant cells and abundant new bone apposition were seen on the β -TCP-implanted defects (Renooji et al. 1985; Neamat et al. 2009).

Despite the promising results in orthopedic surgery (Larsson 2010), β -TCP has provided contradictory evidence in animal (Levin et al. 1974; Barney et al. 1986; Wong et al. 1989; Wada et al. 1989) and human histology studies (Baldock et al. 1985; Dragoo and Kaldahl 1983; Saffar et al. 1990; Froum and Stahl 1987; Stahl and Froum 1986). In dogs, at 16 weeks after the treatment of surgically created three-wall defects, TCP particles were actively resorbed by giant cells and macrophages and were incorporated into new bone matrix (Barney et al. 1986). Similar results were reported in monkeys, where TCP was biodegraded through phagocytosis by histiocytes and multinucleated giant cells. The residual particles were incorporated into the new bone matrix and displayed the bone growth guiding property. In the histological features and immunohistochemical analysis of proliferating cell nuclear antigen (PCNA) of the intrabony defects in dogs augmented with β -TCP, osteoid tissue with a platelike structure and cellular mesenchymal proliferation besides osteoid islands joined by bridges were observed after 3 months. Six months after the implantation, the β -TCP granules were replaced by abundant new platelike bone besides PCNA-enriched, small, oval-shaped mononuclear cells and multinucleated giant cells that were attached to newly formed bones.

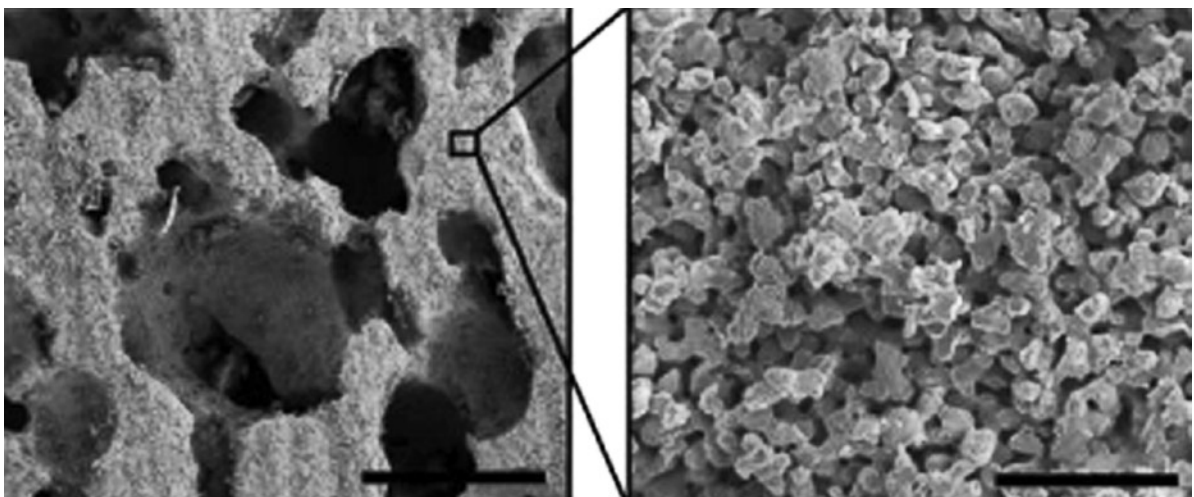


Fig. 2.22 SEM photographs of a commercially available porous β -TCP ceramic (Kamitakahara et al. 2008) (Reprinted with permission from Sage Publications)

No remains of the β -TCP granules could be seen after 3 and 6 months with the newly formed platelike bones and no histological sign of inflammatory reaction or formation of foreign body granulomas (Sugawara et al. 2008). In human histological studies, the TCP particles were encapsulated by fibrous connective tissue, but the particles did not stimulate new bone growth, with residual graft particles evident 18 months following treatment. Although new cementum was observed, there was limited evidence of new attachment (Baldock et al. 1985; Dragoo and Kaldahl 1983; Saffar et al. 1990; Froum and Stahl 1987; Stahl and Froum 1986). Stavropoulos et al. 2010 indicated that the clinical improvements (i.e., PD reduction and CAL gain) obtained after implantation of a granular β -TCP product adjunctively with open flap debridement of periodontal intrabony defects were, in part, characterized by regeneration (although minimal in amount), whereas the major portion of healing occurred with the formation of a long junctional epithelium on the previously affected root surface. In most specimens, β -TCP particles were embedded in the connective tissue, whereas the formation of a mineralized bone-like or cementum-like tissue around the particles was only occasionally observed. In all of the specimens, ghost images of graft particles, appearing as empty spaces due to the decalcification procedure, were observed. The graft particles appeared not to have any apparent association with bone formation, and the major portion of the periphery of the particles was in contact with fibrous connective tissue. A thin layer of a mineralized, occasionally cellular substance in direct contact with some portion of the periphery of the particles was only sporadically observed, and the presence of osteoclasts in contact with the particles was not obvious. The histologic evaluation indicated the formation of new cellular cementum with inserting collagen fibers to a varying extent (mean: 1.9 ± 0.7 mm; range: 1.2–3.03 mm) coronal to the most apical extent of the root instrumentation. The mean new bone formation was 1.0 ± 0.7 mm (range: 0.0–1.9 mm) (Stavropoulos et al. 2010).

Clinical studies reported improvements in clinical outcomes, including clinical attachment level, after the treatment of intrabony periodontal defects involving surgical implantation of β -TCP (Nery and Lynch 1978; Louise et al. 1985; Detienville et al. 1986; Evans et al. 1989; Snyder et al. 1984; Strub et al. 1979; Palti and Hoch 2002; Stavropoulos et al. 2010). Stavropoulos et al. (2010) reported a mean probing depth reduction

from 10.8 ± 2.3 mm presurgically to 4.6 ± 2.1 mm, and a mean clinical attachment level (CAL) gain of 5.0 ± 0.7 mm was observed. The increase in gingival recession was 1.2 ± 3.2 mm.

Bokan et al. (2006) found that the treatment of deep intrabony defects with Emdogain (EMD) either alone or in combination with β -TCP led to a clinically and statistically significant reduction of probing depth (PD) and gain in clinical attachment (CAL). The additional application of β -TCP showed no clear superiority to treatment with Emdogains alone. Treatment with EMD alone yielded a 3.9 ± 1.3 -mm PD decrease and a 3.7 ± 1.0 -mm CAL gain ($P < 0.001$), whereas EMD + β -TCP produced a 4.1 ± 1.2 -mm PD reduction and a 4.0 ± 1.0 -mm PAL gain ($P < 0.001$).

Several commercial available TPC products are available on the market: Bioresorb® is available as porous granulate (particle size: 0.5–2 mm) mainly for dental application. Chronos® and Ceros® (Mathys, Bettlach, Switzerland) are also granular materials with a particle size of 0.5–1.4 mm and pore sizes of 100–500 μ m (60% pore volume), also mainly for dental application. Cerasorb® (Curasan, Kleinostheim, Germany) is available as porous granulate (pore size > 5 μ m) in particle sizes of 0.05–2 mm (grain sizes: 50–150 μ m, 150–500 μ m, 500–1,000 μ m, 1,000–2,000 μ m) for dental application and as machined macroporous blocks for orthopedic applications (Fig. 2.23). Vitoss® is a porous granulate (pore size 10–1,000 μ m; porosity approx. 90%; particle size 3–5 mm) for dental application (Tadic and Eppe 2004). Synthograft™ (Bicon, Boston MA, USA) is available in two particle sizes: 50–500 μ m and 500–1,000 μ m.

Biphasic alloplastic material is produced by sintering hydroxyapatite (HA) and tricalcium phosphate to a chemically united material, with pore sizes of > 100 μ m (Fig. 2.24). Synthetically produced alloplasts used in implant dentistry include Calcitec® Inc. (Austin, TX), Osteogen® (Impladent Ltd, Holliswood, NY), Tricos® (Baxter, Bern, Switzerland), MBCP (Biomatlante, Vigneux de Bretagne, France), Osteon™ (Genoss Co. Ltd., Suwon, Korea) and Bone Ceramic® (Straumann, Basel, Switzerland). However, sufficient documentation of the clinical utility of several of these alloplasts is still lacking (Hallman and Thor 2008). Osteon™ (Genoss Co. Ltd., Suwon, Korea) is composed of 70% HA and 30% β -tricalcium phosphate (β -TCP). HA coated with β -TCP establishes an interconnected scaffold with a porosity of 300–500 nm (Lee et al. 2010).

Ceraform® is a commercially available ceramic, manufactured by Teknimed SA (Vic-en Bigorre, France). This material is a synthetic biphasic ceramic made of 65% HA and 35% TCP. The material is available as a block or granular form and sterilized by gamma radiation. The mean granular diameter is between 900 and

1,200 μm (Develioğlu et al. 2006). BoneCeramic® is a composite of medical purity biphasic calcium phosphate: a mixture of 60% hydroxyapatite, which is 100% crystalline, and 40% of the β form of TCP in particulate form. The graft material is 90% porous with interconnected pores 100–500 μm in diameter.

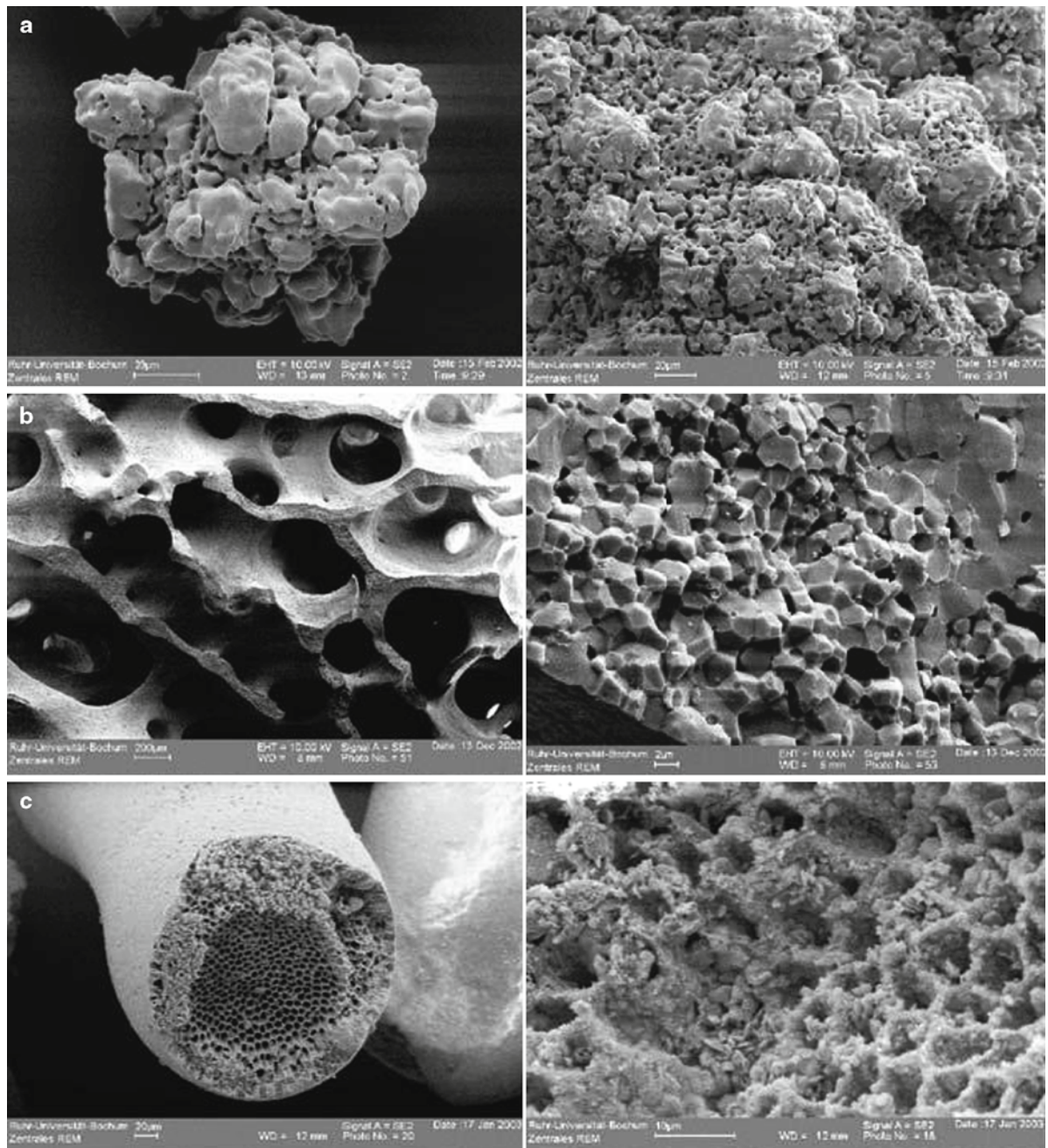


Fig. 2.23 SEM pictures of four representative bone graft materials. (a) Cerasorb®, (b) Cerabone®, (c) Aligpore® and (d) Tutoplast® (bovine) (Tadic and Epple 2004) (Reprinted with permission from Elsevier)

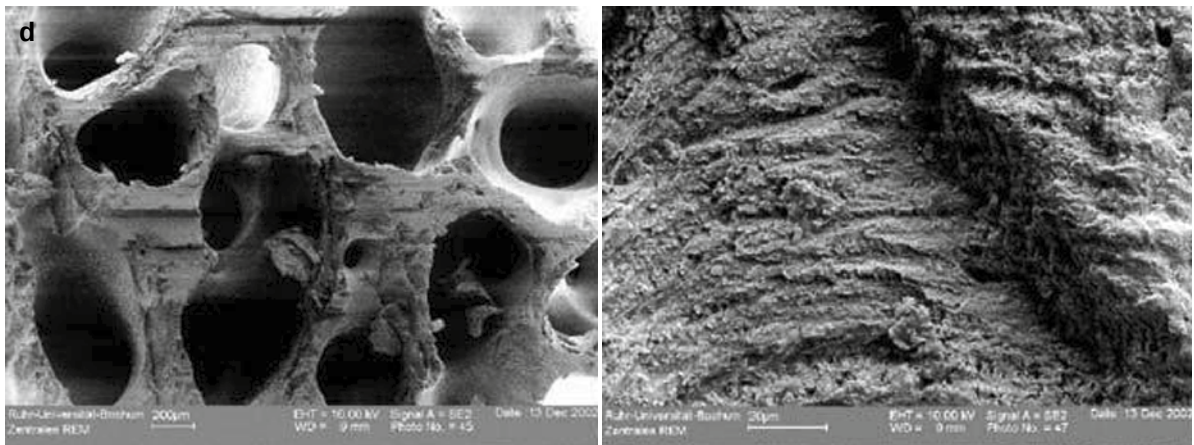


Fig. 2.23 (continued)

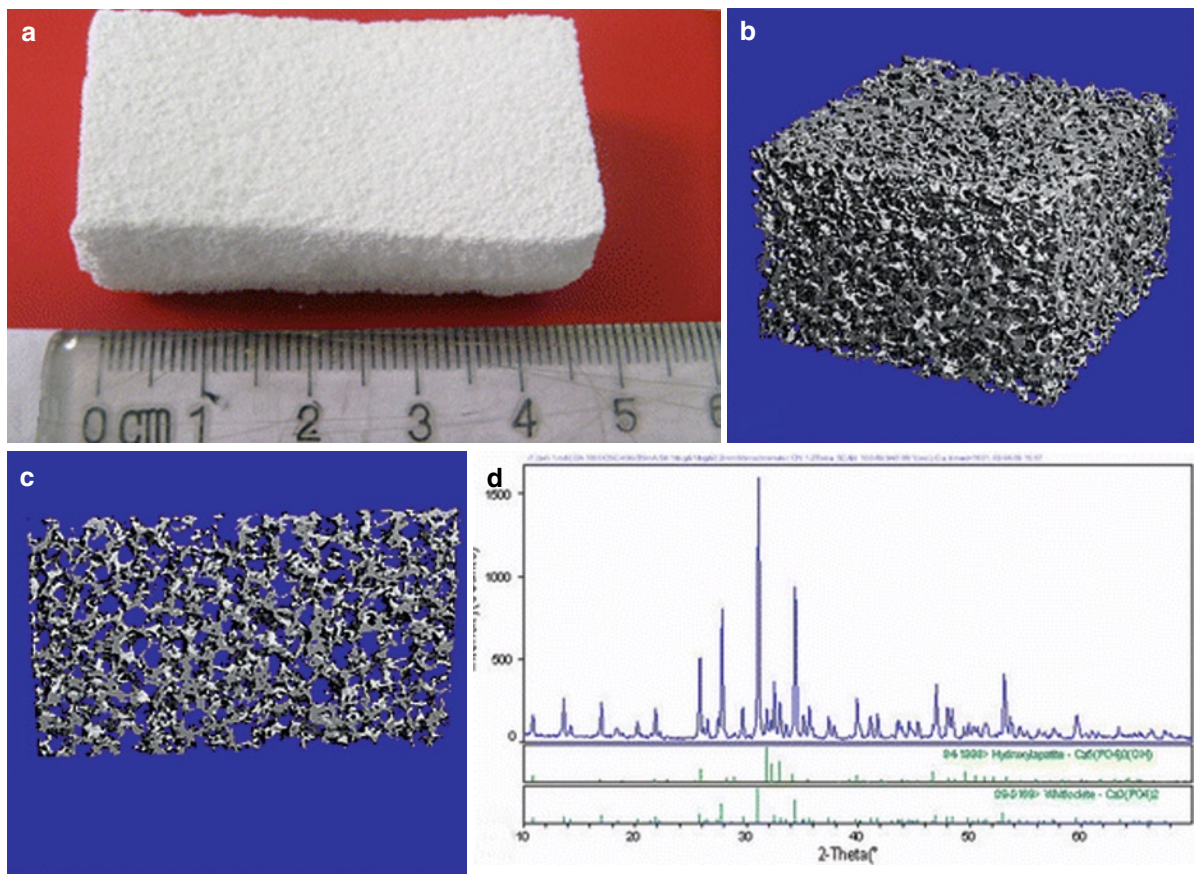


Fig. 2.24 Structural profile of porous biphasic calcium phosphate (PBCP). (a) The aspect of PBCP sample. (b) 3D reconstruction image of BCP by μ CT. (c) Reconstruction transect image of PBCP by μ CT. (d) XRD photograph of PBCP showed that only HA and β -TCP phases were detected. (e) SEM micro-

graphs of macropores in PBCP block ($\times 120$). Bar scales, 1 mm; (f) SEM micrographs of micropores in the wall of macropores, magnified view of (b) ($\times 20,000$). Bar scales, 5 μ m (Wang et al. 2010) (Reprinted with permission from Springer)

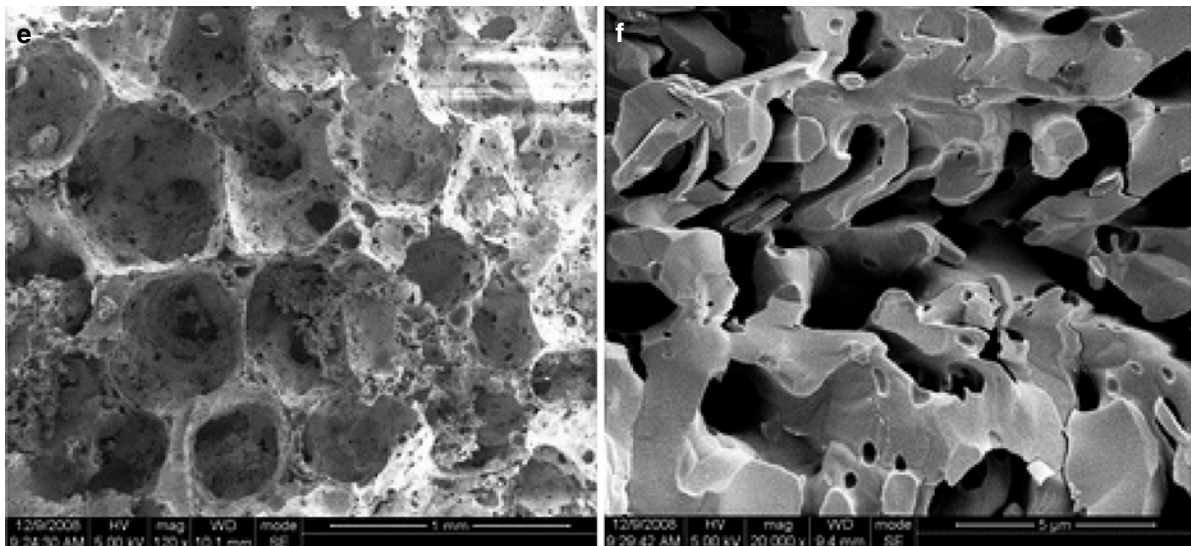


Fig. 2.24 (continued)

Nery et al. (1992) suggested that higher HA ratio showed accelerated new bone formation and new attachment levels and demonstrated the superiority of using a composite of these two materials over the use of either material alone. This grafting material was used in periodontal, peri-implant and various types of bone defects (Gauthier et al. 1999; Piattelli et al. 1996b; Shi et al. 2008; Zafiropoulos et al. 2007; Schwarz et al. 2007; Jensen et al. 2007; Sculean et al. 2008; Wang et al. 2010; Choi et al. 2010) (Fig. 2.25). The combination of Emdogain with a HA/ β -TCP bone substitute did not interfere with the regenerative potential reported for EMD and may result in formation of new cementum with an associated periodontal ligament. However, the combination of Emdogain+HA/ β -TCP resulted in no to minimal new bone formation (Sculean et al. 2008). However, it has been showed that HA/ β -TCP mixed with autogenous spongiosa treatments produced significantly better outcomes than autogenous spongiosa alone in intrabony tissue regeneration (Zafiropoulos et al. 2007).

Some alloplastic materials are mixed together to achieve superior results. Fortoss® Vital (Biocomposites, Staffordshire, UK) is such a mixture of β -TCP and calcium sulfate (Sukumar and Drizhal 2008). Due to modified surface activity and ion loading, its osteoconductive behavior might be superior to conventional calcium phosphates. In contrast to conventional β -TCPs, manufacturing and application of this biphasic calcium composite material use a proprietary process (Zeta Potential Control, Biocomposites) to establish

a negative zeta potential. Based on this concept, the surface of the material will be charged negatively in an aqueous environment (Stein et al. 2009). Zeta potential is an effective predictor of biomaterial attraction to osteoblasts and bone, providing a useful in vitro method for predicting such interactions (Smith et al. 2004; Ohgaki et al. 2001). The application of the β -TCP and calcium sulfate material was well tolerated and led to superior PD and CAL changes compared to open flap debridement for the treatment of intrabony periodontal defects. The clinical benefits of BCC were equivalent to autogenous bone spongiosa (Stein et al. 2009). At 12 months, patients treated with β -TCP and calcium sulfate exhibited a mean PD reduction of 3.6 ± 0.7 mm and a mean CAL gain of 3.0 ± 0.8 mm compared to baseline. Corresponding values for patients treated with autogenous bone spongiosa were 3.4 ± 0.8 mm and 2.9 ± 0.9 mm, whereas open flap debridement sites produced values of 2.8 ± 0.8 mm and 1.6 ± 0.7 mm.

Calcium phosphates can be bound to collagen carriers or mixed with fibrin. The concept is that collagen and fibrin form a network on which minerals can crystallize. Collagen can also bind to extracellular matrix (ECM) proteins of importance in the mineralization process. Healos® (Orquest, Mountain View, CA) is a mixture of hydroxyapatite and bovine collagen and Collagraft® (Zimmer Corp., Warsaw, IN) is composed of 65% hydroxyapatite and 35% tricalcium phosphate combined with bovine collagen. Tricos® is a mixture of hydroxyapatite, tricalcium phosphate and fibrin (Hallman and Thor 2008).

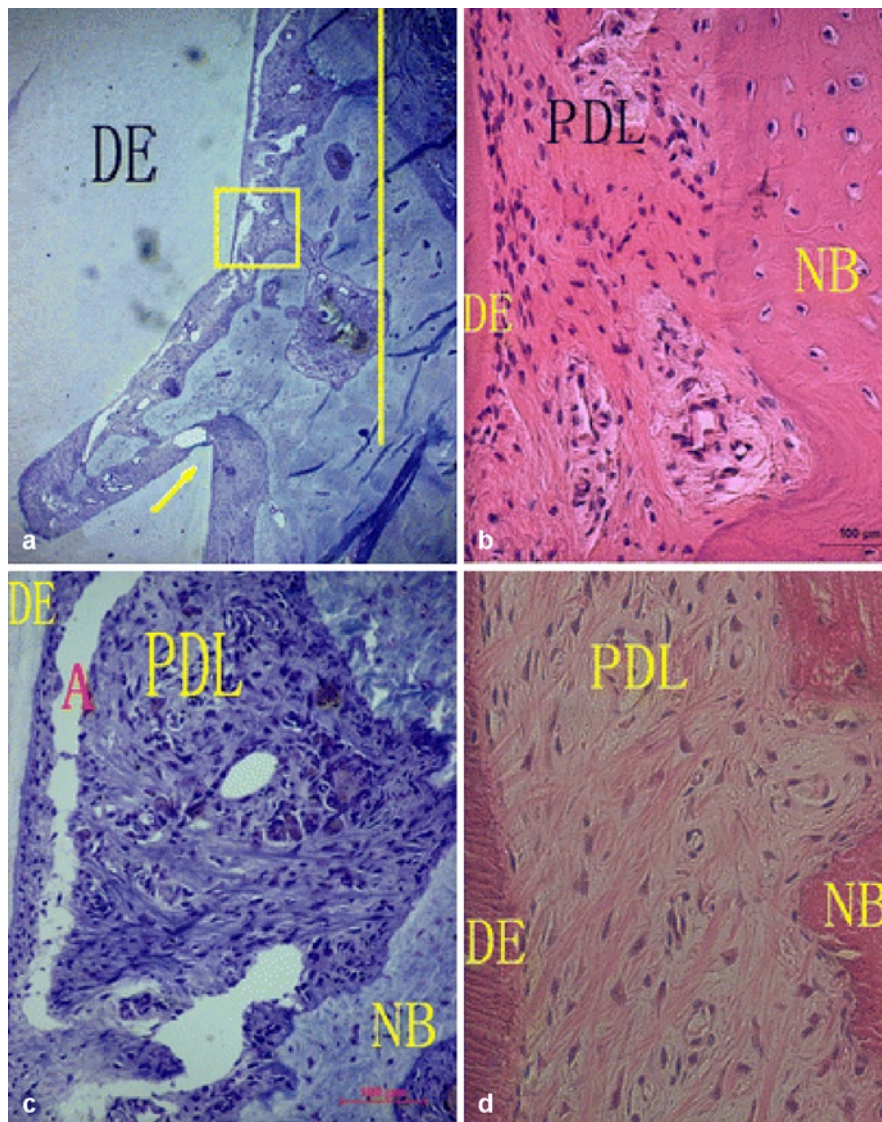
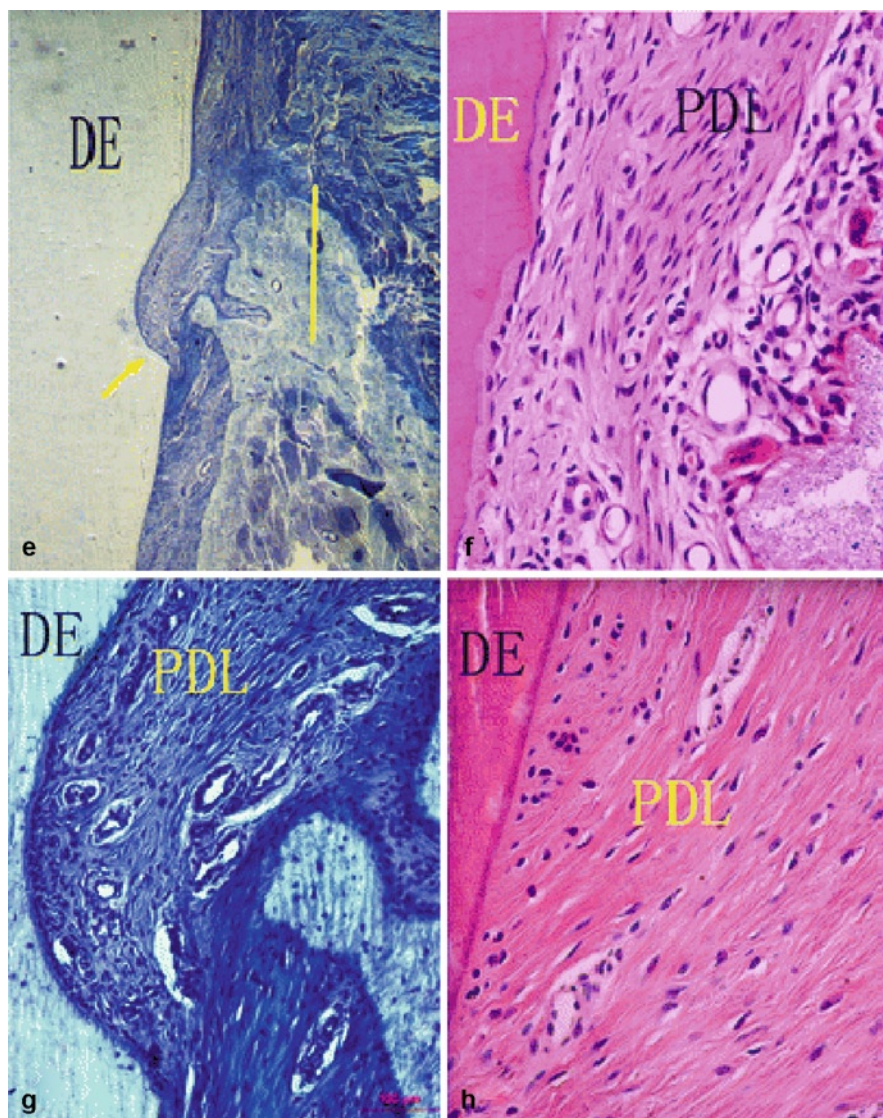


Fig. 2.25 Photomicrograph of healing of acute alveolar bone dehiscence following treatment with porous biphasic calcium phosphate in beagle dogs. The sections of a, c, e and g were stained with *toluidine blue*; the sections of b, d, f and h were stained with *hematoxylin and eosin* (yellow arrows pointed to the notch, yellow lines marked the height of the new alveolar bone, NB new bone, DE dentin, HB host bone, PDL periodontal ligament); (a) 16 weeks in PBCP: healing resulted in abundant periodontal regeneration ($\times 40$); (b) 12 weeks in PBCP: new collagen fiber bundles mostly inclined to the crown with one end embedded in cementum higher than the other end buried in adjacent bone ($\times 400$); (c) 16 weeks in PBCP, magnified view of yellow rectangle in (a): most fiber bundles aligned in vertical to

the root surface ($\times 200$); (d) 24 weeks of PBCP: new collagen fiber bundles were in functional arrangement at right angles ($\times 400$); (e) 16 weeks in OFD: limited new alveolar bone formed in the defect and covered the notch at most ($\times 40$); (f) 12 weeks in OFD: new mixed cementum without inserting collagen fibers deposited on the root surface in the notch, the new PDL were thin and little, which parallel to the root surface ($\times 400$); (g) 16 weeks in OFD, magnified view of (e), newly formed PDL were parallel to the root surface ($\times 200$); (h) magnified view of normal PDL just below the notch, collagen fiber bundles arranged orderly with one end embedded in cementum lower than the other end buried in adjacent bone at about 45° ($\times 400$) (Wang et al. 2010) (Reprinted with permission from Springer)

Fig. 2.25 (continued)

In the recent years, increased efforts have been focused on understanding the mechanisms of and factors required for restoring periodontal tissues in order to increase the predictability of regenerative therapy. These events are controlled by biological mediators like growth factors, morphogenetic proteins, ECM proteins and others, which are produced by monocytes, platelets and resident tissue cells like PDL cells, osteoblasts, cementoblasts and endothelial cells (Christgau et al. 2006). Several studies have shown that periodontal regeneration may be enhanced by the therapeutic application of specific growth factors to tricalcium phosphates, such as basic fibroblast growth factor (bFGF) (Shirakata et al. 2010), growth/differentiation factor-5 (Kwon et al. 2010) and platelet-derived growth factor (PDGF)

(Nevins et al. 2005a; Sarment et al. 2006; McGuire et al. 2006; Nevins et al. 2007; Yassibag-Berkman et al. 2007; Döri et al. 2008; Ridgway et al. 2008; Mellonig et al. 2009; McGuire et al. 2009a; McGuire et al. 2009b).

2.4.6 Calcium Sulfate

Calcium sulfate, generally known as plaster of Paris, or gypsum, is perhaps, the oldest ceramic bone substitute material. Given the relatively simple chemistry of calcium sulfate, there is less latitude for formulation variation than is the case in the calcium phosphate domain. Traditionally, calcium sulfate hemihydrate ($\text{CaSO}_4 \times 1/2\text{H}_2\text{O}$) powder is hydrated to form calcium sulfate

dihydrate ($\text{CaSO}_4 \times 2\text{H}_2\text{O}$), undergoing a slight exothermic reaction to set to a solid form (Eppley et al. 2005).

Calcium sulfate resorbs quickly, over a period of 12 weeks, by a process of dissolution and is substituted by new bone (Bell 1964). The rapid resorption rate can pose a potential problem because the volume of the graft may not be maintained for a sufficiently long period of time to yield reliable grafting results in the esthetic zone (Hallman and Thor 2008).

Calcium sulfate has been considered inexpensive, readily available, easy to sterilize, safe and simple to use, eliciting little or no macrophagic reaction, does not adversely impact the cell proliferation kinetics (Winn and Hollinger 2000; Hogset and Bredberg 1986) and does not elevate serum calcium levels (Elkins and Jones 1988).

Calcium sulfate graft material with a patented crystalline structure described as an alpha hemihydrate acts primarily as osteoconductive bone-void filler that completely resorbs as newly formed bone remodels and restores anatomic features and structural properties. (Nandi et al. 2010). Although the exact mechanism of action remains undiscovered, calcium sulfate appears to function as a resorbable osteoconductive scaffold that provides the structural framework necessary for angiogenesis and osteogenesis while preventing soft tissue invasion by acting as a space filler. It was indicated that calcium sulfate pellets placed into a large animal metaphyseal defect was equivalent to autogenous and allogenic bone in terms of bone-volume production and significantly better than the empty control, as evidenced by backscattered electron microscopy. Histologically, the quality of bone formed in defects treated with calcium sulfate was not discernable from that formed when defects were treated with autogenous or allogenic bone. The histological response was characterized by relative completion of bone formation as evidenced by newly remodeled bone (Peters et al. 2006).

Example of commercially available calcium sulfate graft is Capset®, Lifecore Biomedical, Chaska, MN. CalForma™ Calcium Sulfate Bone Graft Barrier is a modification of Lifecore Biomedical's Capset® Calcium Sulfate Bone Graft Barrier. The modification is the addition of a small amount of an excipient, HPMC (hydroxypropyl methylcellulose or hypromellose), to the accelerated calcium sulfate in order to improve handling characteristics of the device when used as a barrier over bony defects in dental applications (FDA 2010e). Lifecore Biomedical CalMatrix Calcium Sulfate Bone Graft Binder (CalMatrix) is a calcium sulfate material that contains resorbable

surgical grade plaster of Paris with approximately 10% of a pharmaceutical grade sodium carboxymethylcellulose. CalMatrix and Allomatrix® (Wright Medical Technology, Inc) utilize the same calcium sulfate (CS)/CMC blend, except that Allomatrix is provided with human demineralized bone matrix (DBM) already mixed in. CalMatrix is to be mixed with DBM or other bone graft material by the clinician prior to application (FDA 2010e). Currently, medical grade calcium sulfate impregnated with tobramycin is commercially available (Osteoset®; Wright Medical Technology, Arlington, TN, USA) (Sukumar and Drízhai 2008). A novel biphasic calcium composite grafting material that consisted of a porous β -tricalcium phosphate and calcium sulfate phase was recently presented (Fortoss Vital, Biocomposites, Keele, UK).

Potential application of calcium sulfate graft material includes the filling of cysts, bone cavities, benign bone lesions and segmental bone defects; expansion of grafts used for spinal fusion; and filling of bone graft harvest sites. Significant loss of its mechanical properties occurs upon its degradation; therefore, it is a questionable choice for load-bearing applications (Nandi et al. 2010). Another indications include also repair of furcation perforations (Rafter et al. 2002).

Because of its beneficial properties, calcium sulfate alone or associated with other types of materials, such as autologous bone, was used in the treatment of periodontal intrabony defects and furcation lesions (Stein et al. 2009; Paolantonio et al. 2008; Orsini et al. 2008; Harris 2004; Aichelmann-Reidy et al. 2004; Maragos et al. 2002; Orsini et al. 2001; Setya and Bissada 1999; Rosen and Reynolds 1999; Kim et al. 1998; DiBattista et al. 1995; Hashimoto 1983; Shaffer and App 1971).

The addition of DFDBA to calcium sulfate significantly enhanced the clinical outcome more than did the calcium sulfate alone (Maragos et al. 2002) or when compared to surgical debridement only (Setya and Bissada 1999; Kim et al. 1998). The ability to mix calcium sulfate with a graft material, such as DFDBA, has a number of distinct advantages. Conceptually, the calcium sulfate carrier should act as a binder to minimize the graft scatter, facilitate graft retention, decrease graft exposure and maintain the space necessary to permit regenerating tissues to occupy the osseous defect. Aichelmann-Reidy et al. (2004) indicate that calcium sulfate, when used as a binder and barrier in combination with DFDBA, supports significant clinical improvement in intrabony defects, as evidenced by reductions in probing depth, gains in clinical attachment level and defect fill and resolution. Calcium

sulfate represents an important alternative to non-resorbable ePTFE barriers in combination with DFDBA for the treatment of intrabony defects, with less morbidity and cost for the patient (Aichelmann-Reidy et al. 2004). No significant differences were seen between calcium sulfate graft and membrane versus guided tissue regeneration with collagen membrane alone (Paolantonio et al. 2008) or between the results obtained using the combination of autogenous bone grafting plus calcium sulfate or autogenous bone grafting with a bioabsorbable membrane (Orsini et al. 2008; Orsini et al. 2001).

Some alloplastic materials are mixed together to achieve superior results. Fortoss® Vital (Biocomposites, Staffordshire, UK) is such a mixture of beta tricalcium phosphate and calcium sulfate (Sukumar and Drizhal 2008).

2.4.7 Bioactive Glasses (BG)

Among the different alloplastic materials used in periodontal therapy, hydroxyapatite, calcium phosphates and bioactive glass ceramics share a common factor, which is their capacity to form a carbonated hydroxyapatite layer on their surfaces once exposed to simulated body fluids or implanted in vivo, hence the concept of “bioactivity.” Since their invention three decades ago by Hench et al. (1971) bioactive glasses have clinically gained wide acceptance in restorative orthopaedics and dentistry (Hattar et al. 2005).

The original composition of bioactive glass approved by the FDA, designated 45 S5, was composed of 46.1 mol% SiO_2 , 26.9 mol% CaO , 24.4 mol% Na_2O , and 2.5 mol% P_2O_5 . The original composition and fine structure has been extensively modified in an attempt to further enhance bioactive glass as a bone replacement graft (Reynolds et al. 2010; Hench 2006). When a bioactive glass is implanted in vivo, the pH of the site increases close to 10, a layer rich in silica gel is formed on the surface of the particles and a subsequent calcium phosphate layer is formed by the interaction between calcium and phosphate from the bioactive glass and tissue fluids. The calcium phosphate layer is composed of hydroxycarbonate apatite that is chemically and structurally equivalent to bone mineral composition (Villaça et al. 2005; Hench & Wilson et al. 1984) (Fig. 2.26).

The material is *easy to manipulate* and it is *hemo-static* (Wilson and Low 1992; Low et al. 1997; Zamet et al. 1997; Froum et al. 1998; Lovelace et al. 1998; Sculean et al. 2002a). The bioactive glass particles

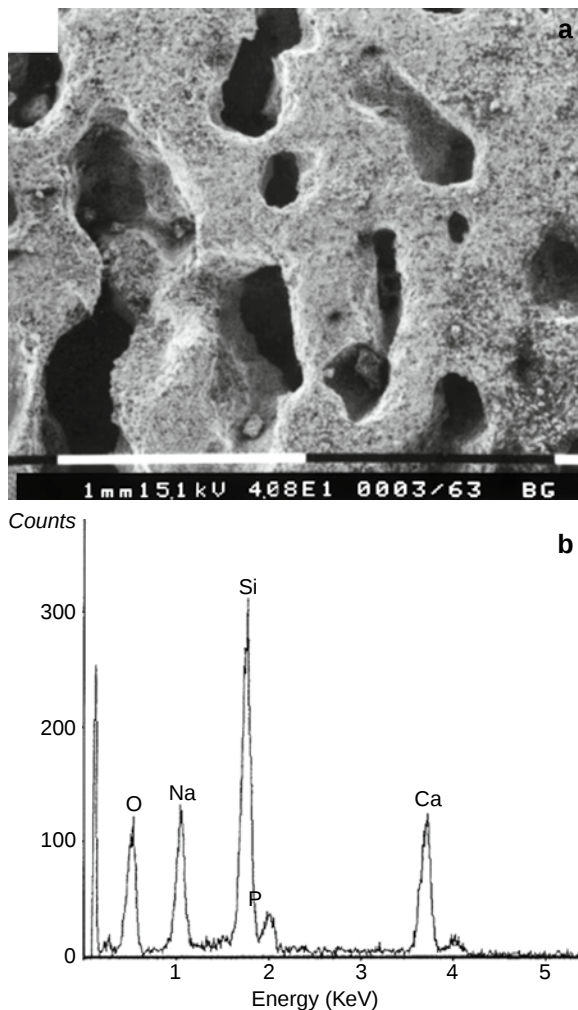


Fig. 2.26 Porous structures and chemical composition of glass ceramic: (a) porous structures of glass ceramic under SEM observation and (b) EDX analysis of glass ceramic (Yuan et al. 2001) (Reprinted with permission from John Wiley & Sons)

formed a cohesive mass when wetted with blood, which allowed very easy manipulation and packing into the extraction sockets or periodontal defects (Schepers et al. 1998). This transparent bioactive material has proven ability to *bond to connective tissue and bone* without an intervening fibrous connective tissue interface (Wilson and Low 1992). Upon contact with body fluid, there is an immediate exchange of ions which results in a physiochemical bond between Bioglass, soft tissue and bone. The ion exchange creates an environment resulting in the formation of a hydroxyl-carbonate apatite layer (HCA), a biological apatite identical to the mineral phase of bone, which allows for more rapid repair and regeneration of bone than other synthetic graft materials (Wilson and Low 1992; Shapoff et al. 1997).

It was showed that bioactive glass several **antibacterial effect** against on a large panel of clinically important bacterial species (*A. actinomycetemcomitans*, *P. gingivalis*, *Actinomyces naeslundii*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Streptococcus mutans*, and *Streptococcus sanguis*, *Candida albicans*) (Stoor et al., 1998; Allan et al., 2001; Allan et al., 2002; Yli-Urpo et al., 2003; Zehnder et al., 2004; Munukka et al., 2008; Hu et al., 2009).

Bioglass is capable of promoting osteoblast cellular proliferation and differentiation (Price et al. 1997; Xynos et al. 2000a; Vrouwenvelder et al. 1993; Xynos et al. 2000b; Hattar et al. 2005; Palmieri et al. 2008) and acts on bone formation by determining both osteoconduction (as demonstrated by the reduced cell adhesion) and osteogenesis (as shown by TGF β -related proteins and stem cell markers) (Carinci et al. 2007). Bioactive glass is a particulate bioactive ceramic, which has the ability to bond to bone tissue and enhance bone growth because of its *osteoconductive properties*. In addition to its osteoconductive properties, it also has an *osteostimulatory effect* showing bone growth within eroded particles. These islands of newly formed bone tissue function as nuclei for further bone growth and enhance the repair of osseous defects. This new bone has the histologic and biomechanical properties of surrounding bone as soon as 7 months after grafting (Furusawa et al. 1998; Thronsdon and Sexton 2002) (Fig. 2.27).

Treatment of two-wall intrabony defects in monkeys demonstrated that bioactive glass had better healing potential than debridement only. Bioactive glass showed an inhibitory property on the apical migration of the junctional epithelium. It was observed that in the sites treated with the bioactive glass, the junctional epithelium migrated apically to the level of the particles most coronally located inside the defect, not surpassing this point (Villaça et al. 2005). Karatzas et al. (1999) in a histological study in monkeys reported significantly more new cementum and less epithelial downgrowth in the sites that received bioactive glass. However, in human histological studies, a low potential to facilitate periodontal regeneration was demonstrated as there was minimal new bone formation limited to the most apical borders of the defects. No signs of periodontal regeneration as defined by new cementum, periodontal ligament and bone formation on a previously diseased root surface were observed (Nevins et al. 2000; Sculean et al. 2005c).

In treatment of periodontal intrabony osseous defects or furcation lesions, Bioglass was used either alone (Froum et al. 1998; Ong et al. 1998;

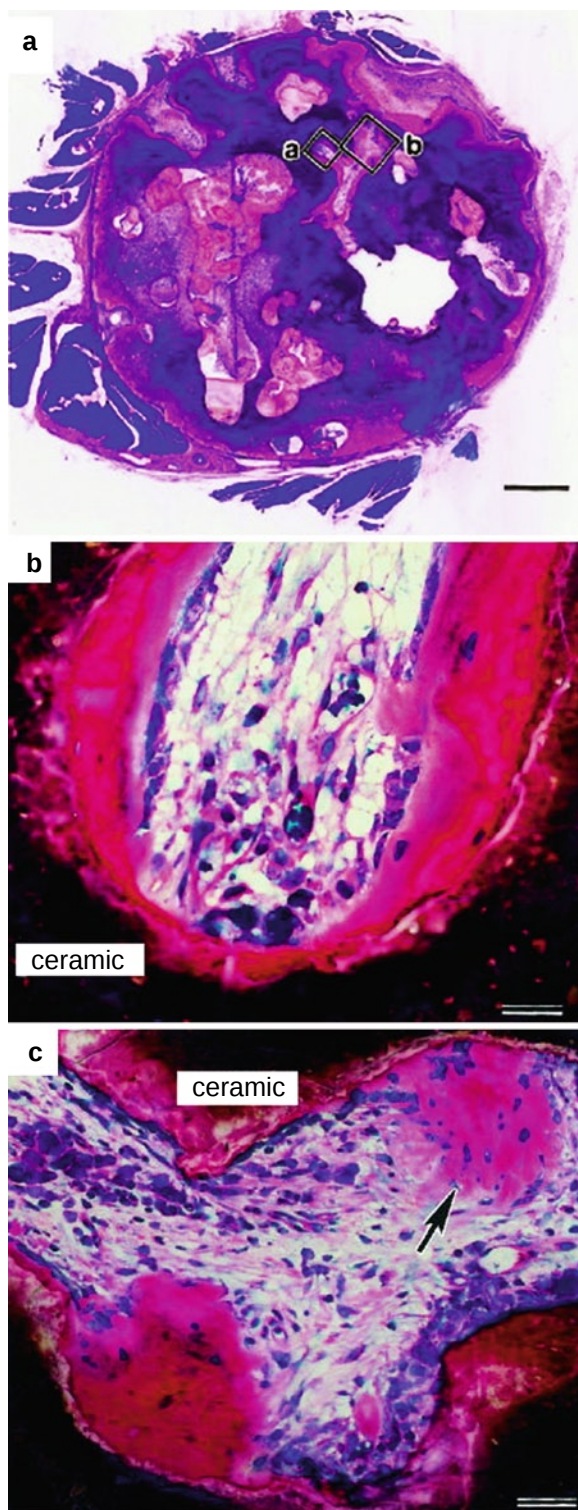


Fig. 2.27 Histological observation of bone formation induced by glass ceramic: (a) an overview of a histological section (bar = 700 μ m), (b) a high magnification of square a in (a) (bar = 50 μ m), (c) a high magnification of square b in (a) (arrow: intramembranous ossification, bar = 100 μ m) (undecalcified section, methylene blue and basic fuchsin staining) (Yuan et al. 2001) (Reprinted with permission from John Wiley & Sons)

Rosenberg et al. 2000; Park et al. 2001; Zamet et al. 1997; Anderegg et al. 1999; Yukna et al. 2001; Park et al. 2001; Mengel et al. 2003, 2006; Dybvik et al. 2007; Leknes et al. 2009), with GTR membranes (Keles et al. 2006), with platelet-rich plasma (Demir et al. 2007a, 2007b) or with enamel matrix derivative (EMD) (Sculean et al. 2002a, 2005b, 2005c, 2007a; Kuru et al. 2006).

Treatment of intraosseous defects by means of bioactive glass resulted in an improvement of the bony lesion when compared to the OFD procedure. Weighted mean difference in clinical attachment level gain between bioactive glass and OFD was reported to be 1.04 mm (95% CI: 0.31–1.76) by Trombelli et al. (2002) and 1.05 ± 1.89 mm by Reynolds et al. (2003), while for the PD change, the weighted mean difference was 0.6 mm (95% CI: 0.20–1.002) (Trombelli et al. 2002), and $0.71 \text{ mm} \pm 2.22$ mm (Reynolds et al. 2003), respectively.

Leknes et al. (2009) examined the clinical efficacy of EMD and BG in the treatment of proximal intrabony periodontal defects and to evaluate factors influencing the treatment outcome. The gain in proximal attachment after treating intrabony defects by flap surgery with BCF was significant ($P=0.004$) and twice that following treatment with EMD ($P=0.056$). Patient and site variables affected the clinical outcome differently. Regression analysis revealed that within the EMD group, smoking and tooth mobility negatively influenced the gain of attachment, whereas within the BG group, gingival recession increased with age, increasing cemento-enamel junction to buccal crest distance and increasing mesial-distal width of the defect.

The combination of enamel matrix derivative and bioactive glass does not seem to additionally improve the clinical results when compared with enamel matrix derivative alone (Sculean et al. 2002a; Sculean et al. 2005b; Sculean et al. 2007a). Kuru et al. (2006) reported for both treatments marked clinical and radiographical improvements, but showing that combined treatment seemed to enhance the results in the treatment of wide intrabony defects. The two groups, EMD and EMD + BG, presented a mean pocket reduction of 5.03 ± 0.89 and 5.73 ± 0.80 mm, recession of 0.97 ± 0.24 and 0.56 ± 0.18 mm, relative attachment gain of 4.06 ± 1.06 and 5.17 ± 0.85 mm, and radiographic bone gain of 2.15 ± 0.42 and 2.76 ± 0.69 mm,

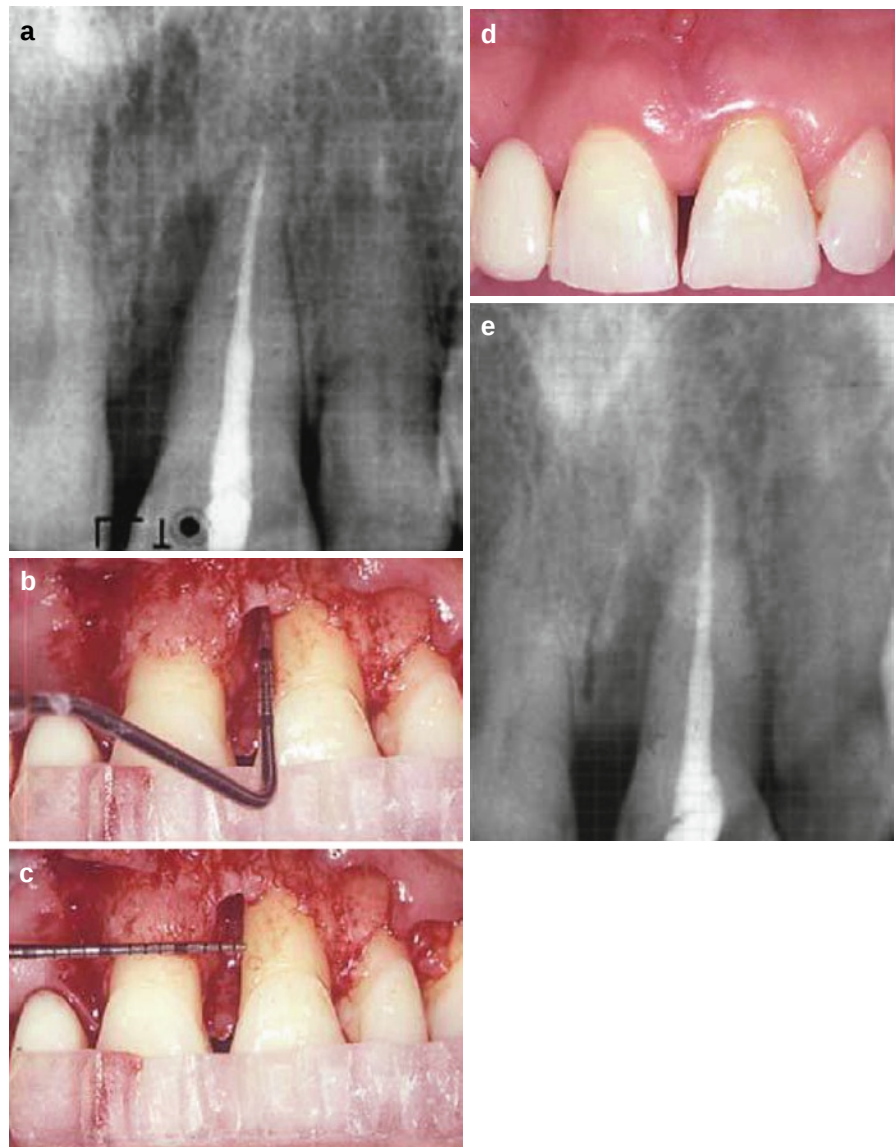
respectively. It was indicated that the clinical improvements obtained with both regenerative modalities can be maintained over a period of 4 years (Sculean et al. 2007a) (Figs. 2.28 and 2.29).

Platelets contain high concentrations of PDGF and TGF- β in their α granules and the preparation of platelet-rich plasma (PRP) seems to be an appropriate and economical method to obtain these growth factors autogenously (Demir et al. 2007a). Demir et al. (2007b) compared the use of either PRP + BG to BG alone in the treatment of patients with interproximal probing depth ≥ 6 mm following initial therapy. Clinical parameters were recorded at baseline and repeated 9 months after surgery and surgical reentries were also performed. The results showed that both treatment modalities were effective. Pocket depth reduction of 3.60 ± 0.51 mm, clinical attachment gain of 3.3 ± 1.77 mm and defect fill of 3.47 ± 0.53 mm were noted in the PRP/BG group, with 3.29 ± 1.68 , 2.86 ± 1.56 and 3.36 ± 0.55 mm improvements, respectively, noted for the BG group. None of the differences between the two treatment modalities were statistically significant.

When the long-term effectiveness of a bioabsorbable membrane and a bioactive glass in the treatment of intrabony defects in patients with generalized aggressive periodontitis was performed, highly significant improvements in the parameters PD and CAL were recorded after 5 years with both regenerative materials. Reduction in PD of 3.6 ± 0.8 mm ($P=0.016$) and a gain in CAL of 3.0 ± 2.0 mm ($P=0.01$) were registered in the membrane group. There was a slight increase in GR by 0.6 ± 1.4 mm ($P=0.334$). In the PG group, a reduction in PD of 3.5 ± 1.4 mm ($P=0.01$) and a gain in CAL of 3.3 ± 2.1 mm ($P=0.01$) were recorded, whereas GR increased by 0.2 ± 1.7 mm ($P=0.525$). Radiographically, the defects were found to be filled significantly more in the bioactive glass group (Mengel et al. 2006). Equal clinical results with bioactive glass and ePTFE barriers in mandibular molar Class II furcations were obtained (Yukna et al. 2001). However, bioactive glass was associated with simpler application and required no additional material removal procedures.

Moreover, in soft and hard tissue measurements, no significant differences were reported between demineralized freeze-dried bone allografts (DFDBA) and BG grafted sites (Lovelace et al. 1998). The results indicated that probing depths were reduced by 3.07 ± 0.80 mm with the bioactive glass and

Fig. 2.28 Enamel matrix derivative in combination with a bioactive glass in wide intrabony defects. **(a)** A preoperative radiograph revealing the presence of an intrabony defect. **(b)** Intrabony component of the defect. **(c)** The horizontal width of the defect. **(d)** The clinical appearance of the defect filled with the combination of enamel matrix derivative and bioactive glass at 8 months. **(e)** Radiographic appearance at 8 months postoperatively (Kuru et al. 2006) (Reprinted with permission from Springer)



2.60 ± 1.40 mm with DFDBA. Sites grafted with bioactive glass resulted in 2.27 ± 0.88 mm attachment level gain, while sites grafted with DFDBA had a 1.93 ± 1.33 mm gain in attachment. Bioactive glass sites displayed 0.53 ± 0.64 mm of crestal resorption and 2.73 mm bone fill. DFDBA-grafted sites experienced 0.80 ± 0.56 mm of crestal resorption and 2.80 mm defect fill. The use of bioactive glass resulted in 61.8% bone fill and 73.33% defect resolution. DFDBA-grafted defects showed similar results, with 62.5% bone fill and 80.87% defect resolution. Both treatments pro-

vided soft and hard tissue improvements when compared to baseline ($P \leq 0.0001$).

The significant base of scientific studies conducted using Bioglass, along with the required biocompatibility and toxicology studies required by the regulatory bodies have provided a strong basis for establishing the safety of Bioglass devices placed into commerce (Hench 2006).

While the second-generation Bioglass materials performed admirably in replacing diseased or missing hard tissue, the discoveries that Bioglass could

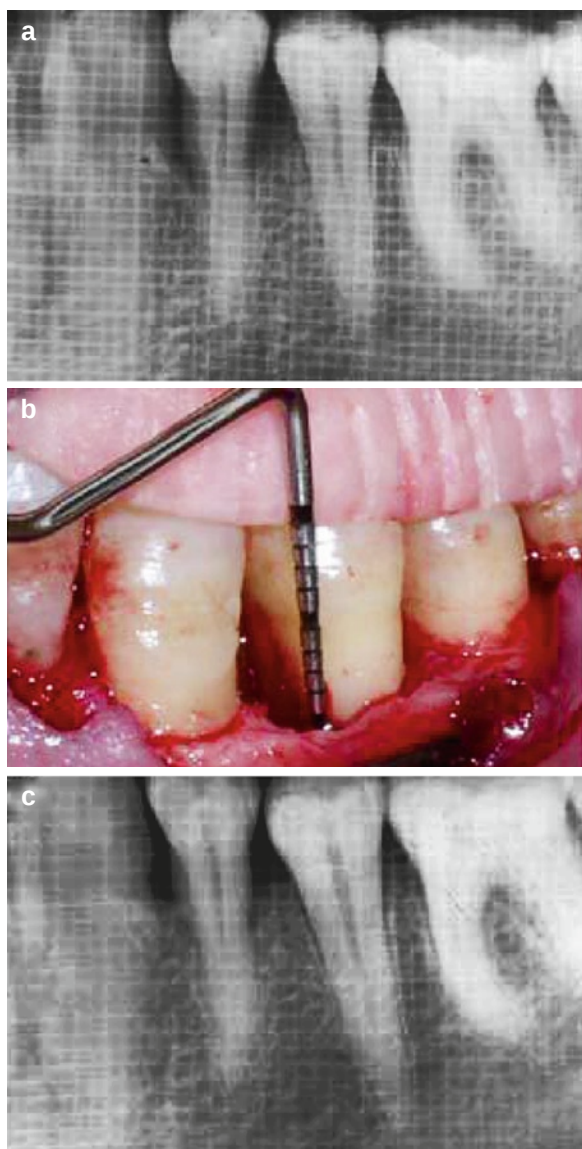


Fig. 2.29 Enamel matrix derivative alone in wide intrabony defects. (a) A preoperative radiographic appearance. (b) Intrabony component of the defect. (c) At 8 months after treatment with enamel matrix derivative (Kuru et al. 2006) (Reprinted with permission from Springer)

positively affect osteoblasts, and in fact “stimulate” them to produce more bone tissue earlier than other synthetic biomaterials led to the concept of “osteoproduction” and “osteostimulation.” In order to take advantage of this property, and of the need to regenerate diseased or missing tissues, the development of third-generation Bioglass products focused on

using particles rather than monolithic shapes. The products are being manufactured and sold to the clinic under the name NovaBone (Hench 2006).

The first NovaBone particulate material cleared for sale in the USA was Perioglas®, which was cleared via the 510[k] process in December, 1993. In 1995, PerioGlas obtained a CE Mark and marketing of the product began in Europe. The initial indication for the product was to restore bone loss resulting from periodontal disease in infrabony defects. In 1996, additional indications for use were cleared by FDA, including use in tooth extraction sites and for alveolar ridge augmentation (Hench 2006).

PerioGlas® (Block Drug Co., NJ, USA) is a synthetic absorbable osteoconductive bone graft substitute composed of a calcium phosphosilicate bioactive glass, Bioglass. The device is in a particulate form of a size range 90–710 μm . The device is intended for dental intraosseous, oral and cranio-/maxillofacial bony defects. It is supplied sterile, packaged either in a Tyvek-sealed PET-G cup or in a filled syringe within a second sterile barrier package. The device packages are protected by an outer shrink-wrapped cardboard box. At time of use, the device is mixed with sterile water, saline, the patient’s own blood or marrow or with autogenous or allograft bone to form a wet sandy paste that is applied to the defect (FDA 2010a).

PerioGlas® Plus (Block Drug Co., NJ, USA) is a synthetic resorbable osteoconductive bone graft substitute composed of a calcium phosphosilicate material and a calcium sulfate binder. The device is intended for dental intraosseous, oral and maxillofacial bony defects. The inorganic calcium and phosphorous components are thermally incorporated in a sodium silicate network (*PerioGlas®*) designed specifically for its absorbability and osteoconductive nature. The calcium sulfate component binds the *PerioGlas®* particles together at the time of implantation and is absorbed from the graft site over the first several weeks following implantation. On absorption of the calcium sulfate, the *PerioGlas®* particles remain in the graft site and are progressively absorbed and replaced by host bone during the healing process (FDA 2010b).

The main technological characteristic difference between *PerioGlas® Plus* and the predicate devices is their composition. *PerioGlas®* is composed of particulate Bioglass. Capset (Lifecore Biomedical,

Chaska, MN) is composed of powdered calcium sulfate hemihydrate which, when combined with an aqueous-based setting solution, is chemically converted to calcium sulfate dihydrate. PerioGlas® Plus is composed of particulate Bioglass and powdered calcium sulfate hemihydrate; when mixed with water, the hemihydrate is chemically converted to calcium sulfate dihydrate and acts as a binder for the Bioglass particles. The calcium sulfate in the PerioGlas® Plus and Capset devices is absorbed between 4 and 8 weeks after implantation, depending on the graft site, size and material used. The particulate Bioglass in the PerioGlas® Plus device is identical to that in the PerioGlas® predicate, being substantially absorbed within the 6-month time frame normally associated with bone remodeling. For all three devices, bone forms throughout the graft site with the material being absorbed and replaced by new bone tissue (FDA 2010b).

Biogran™ (Orthovita Inc., Malvern, PA, USA) is a particulate bioactive glass, which has been used in a few experimental and clinical studies for the treatment of different types of bone defect. BioGran™ is a resorbable bone graft material consisting of 300–355 µm diameter bioactive glass particle size. It has the ability to bond to soft and bone tissue and, in addition, it enhances bone tissue growth due to its osteoconductive properties (Schepers et al. 1991, 1993; Kontonasaki et al. 2007).

Unigraft® (Unicare Biomedical Inc., Laguna Hills, CA, USA) is a synthetic bioactive glass that is intended to use in the repair oral/maxillofacial and dental intraosseous defects. The bioactive glass (CaO, Na₂O, P₂O₅ and SiO₂) used in Unigraft® is manufactured as irregular-shaped synthetic granules, sized from about 200 µm to about 420 µm. It is supplied sterile in foil-sealed polyolefin vial. The product is to be mixed with sterile saline or with patient's blood to form a sandy paste that is to be applied to the defect (FDA 2010c).

More recently, Bioglass particulate has been used for the treatment of dentinal hypersensitivity. Tooth hypersensitivity is a problem that affects an estimated 15–20% of the population of the USA, and similar numbers in Europe. Tooth hypersensitivity occurs when the root portion of the tooth, which is dentin, becomes exposed around the gum line. The dentin has small openings, or tubules, that communicate with the

pulp chamber. If the dentinal tubules become exposed, hot or cold or pressure can transmit the sensations to the nerves in the pulp, causing pain. The Bioglass material used in this application is a very fine particulate that is incorporated into toothpaste, or used with an aqueous vehicle and applied to the tooth surface around exposed root dentin. When Bioglass particles are put in contact with dentin, they adhere to the surface, rapidly form a hydroxycarbonate apatite layer and occlude the tubules, thereby relieving the pain (Hench 2006). Studies have shown that the Bioglass particulate could produce considerable sealing depth in dentinal tubules with the potential of prolonging the therapeutic effect efficaciously (Curtis et al. 2010; Chiang et al. 2010; Lee et al. 2007; Lee et al. 2005; Kuo et al. 2007).

Other dental and maxillofacial applications include pulp capping (Stanley et al. 2001; Oguntebi et al. 1993), for bone formation in combination with implants and sinus lift procedures (Govindaraj et al. 1999; Browaeys et al. 2007; Precheur et al. 2007), including its use in solid root form in extraction sockets as endogenous ridge maintenance implants (Stanley et al. 1987; Wilson et al. 1993a; Kirsh and Garg 1994), for filling oral cystic cavities or after apicoectomies (Shapoff et al. 1997).

2.4.8 Oily CaOH₂ Suspension

Calcium hydroxide (CaOH₂) is a product of lime slaking from quicklime (CaO) and is used extensively in endodontics, combined with various vehicles for indirect and direct pulp-capping procedures and as a temporary root-filling material, where it has been shown to support hard tissue repair. Recently, a non-setting oily CaOH₂ suspension (OCHS; Osteoinductal®, Osteoinductal GmbH, Munich, Germany) has been introduced into the market for application in jaw-bone surgery. This formulation contains, apart from CaOH₂, *liquid and solid carbohydrate chains and various fatty acids (e.g., oleic, palmitoleinic, gadoleinic, margarine, pentadecane, myristic, linolenic, stearic, arachidic, lauric) esterified with glycerol, while the oily part consists of a natural product of porcine origin, oleum pedum and vaselinum album* (Stavropoulos et al. 2007).

Results from pilot studies in *experimental animals* suggest that OCHS may accelerate bone healing and promote periodontal regeneration (Ito et al. 2002; Schwarz et al. 2006b). In a study in rats, Ito et al. (2002) compared the healing in OCHS-filled extraction sockets with that in sockets left untreated to serve as controls. The authors reported that after 1 month of healing, the sockets previously filled with OCHS showed statistically significantly larger amounts of bone fill than the controls. In dogs, three-wall intrabony periodontal defects were bilaterally produced and were randomly treated with both access flap surgery and the application of OCHS or access flap surgery alone. After 2 months of healing, larger amounts of regenerated bone and newly formed cementum were observed in the sites treated with OCHS than that found in the control sites, in which healing was predominantly characterized by the formation of a long junctional epithelium along the previously denuded root surface and only minimal bone regeneration (Schwarz et al. 2006b) (Fig. 2.30). In contrast with the previous reports, Stavropoulos et al. (2007) clearly demonstrated in an experimental study in rats that

OCHS does not promote bone formation when used as an adjunct to GBR, but on the contrary it may hamper it. It was also indicated that the use of the calcium hydroxide suspension. Osteoinductal has a detrimental effect on wound healing and osseointegration of dental implants and cannot be recommended for use with dental implants (Kohal et al. 1997). An in vitro study indicated that Osteoinductal enhances the mitogenic response of human PDL cells by activation of ERK1/2 and increases cell proliferation; however, it is inferior in comparison to EMD (Kasaj et al. 2007).

Recent clinical studies have shown that an oily calcium hydroxide suspension, applied to the root surface in conjunction with surgical periodontal therapy, may promote periodontal regeneration (Stratul 2003; Stratul and Sculean 2004; Stratul et al. 2006). OCHS resulted in statistically significant higher pocket depth reductions and clinical attachment level gains than access flap surgery alone. At 6 months after surgery, the test group showed a reduction in mean PD from 7.7 ± 1.5 to 2.9 ± 0.9 mm ($P < 0.001$) and a change in mean CAL from 9.6 ± 2.1 to 5.5 ± 2.5 mm ($P < 0.001$). In the control group, the mean PD was reduced from

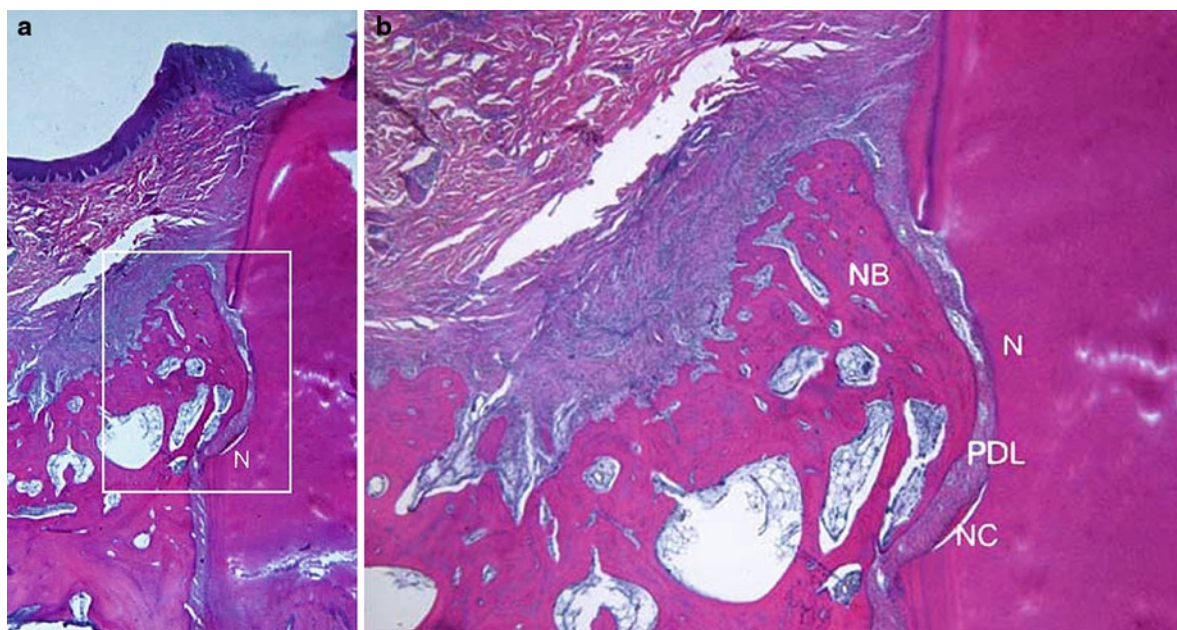


Fig. 2.30 Effect of an oily calcium hydroxide suspension (Osteoinductal) on healing of intrabony periodontal defects in dogs. (a) Histological view of specimen 1 following application of oily calcium hydroxide suspension (OHCS) showing periodontal regeneration (magnification $\times 1.25$). (b) Higher magnifi-

cation of the regenerated area shown in a demonstrated formation of cementum, periodontal ligament and alveolar bone (magnification $\times 4$) (Schwarz et al. 2006b) (Reprinted with permission from Springer)

6.9 ± 0.9 to 3.7 ± 0.9 mm ($P < 0.001$) and the mean CAL changed from 8.5 ± 2.5 to 6.4 ± 2.7 mm ($P < 0.001$) (Stratul et al. 2006).

It has been also shown that the topical subgingival application of an oily calcium hydroxide suspension (Osteoinductal) after nonsurgical periodontal therapy improved early periodontal wound healing. The application of Osteoinductal resulted in significantly greater improvement in gingival and bleeding indices in experimental versus control sites at the 1-, 2- and 3-week examinations. In addition, no side effects like inflammation or pain were reported at sites subjected to Osteoinductal application (Kasaj et al. 2006).

2.4.9 Porous Titanium Granules

Recently, a non-resorbable, osteoconductive bone substitute, was proposed to be used in stabilization of hip prostheses (Alffram et al. 2007; Turner et al. 2007), in connection with surgery of compression fractures in the lateral tibial plateau (Jónsson and Mjöberg 2009), in cases with large cystic cavities (Magistri et al. 2006), in patients planned for augmentation of the sinus floor prior to or in conjunction with placement of dental implants (Holmberg et al. 2008; Bystedt and Rasmusson 2009; Bystedt 2007) and in the surgical treatment of peri-implant osseous defects (Wohlfahrt et al. 2010c; Frei et al. 2010; Bergmann 2010).

Tigran™ PTG (Natix, Tigran Technologies AB, Malmö, Sweden) is irregularly shaped and porous granules manufactured using commercially pure titanium. The granules are between 0.7 mm and 1.0 mm. When they are mixed with the patient's blood or with a saline solution, the granules attach to each other due to the capillary force. The titanium surface is very thrombogenic, which facilitates the formation of stabilizing blood clots around the granules. The granules that have a porosity of about 80% and an osteoconductive surface structure, imitate properties of human bone, and create a scaffolding for bone generation that stimulates osteoblast colonization and osseointegration. The granules are non-resorbable and keep their volume during the operation and the entire healing period which ensures mechanical stability and a desired aesthetic result. Tigran™ PTG is easy to use. No special tools are needed. When osseointegration is completed, common drilling techniques are used when an implant has to be placed in the treated area (<http://www.tigran.se/>

[en/professional/products/tigran-tm-ptg/](http://www.tigran.se/en/professional/products/tigran-tm-ptg/)). The titanium granules do not set (i.e., no risk of heat injury to the bone) and can therefore be handled without time pressure during surgery (Jónsson and Mjöberg 2009).

Tigran's porous titanium granules have been demonstrated experimentally to have superior microstructural properties (porosity, interconnectivity, open pore size and surface area-to-volume ratio), cell viability and proliferation rate compared to both Straumann BoneCeramic and Geistlich Bio-Oss (Sabatrasekh et al. 2010).

Histological examination in an animal model revealed, 6 months after implantation, lamellar bone formation through the mantle of porous titanium granules in continuity with the surrounding cortex resulting in the formation of an integrated mantle of bone and titanium granulate around the prosthesis (Turner et al. 2007). When used calibrated defects prepared in the tibias of New Zealand rabbits, both metallic and oxidized porous titanium granules demonstrated osteoconductive proprieties that can be used to promote bone formation in osseous defects adjacent to titanium implants without hampering implant osseointegration (Wohlfahrt et al. 2010a). A randomized animal experiment has indicated that degree II furcation defects in minipigs grafted with PTG demonstrated significantly better osseous regeneration compared bovine hydroxyapatite (Bio-Oss) grafted defects. No significant signs of adverse events were seen in any of the treatment groups (Wohlfahrt et al. 2010b).

No randomized clinical trials are presently available regarding the efficacy of porous titanium granules in the treatment of periodontal defects.

2.5 Composite Grafts

One of the most promising emerging surgical options may be the use of a "composite graft" that contains osteogenic cells and osteoinductive growth factors along with a synthetic osteoconductive matrix. Composite materials being tested in preclinical and clinical trials may exhibit functionality comparable to autograft and allograft. Composite synthetic grafts offer an alternative that can potentially unite the three essential bone-forming properties in more controlled and effective combinations without the disadvantages found with autograft. A composite graft combines an osteoconductive matrix with bioactive agents that provide osteoinductive and osteogenic properties, potentially replicating autograft functionality. The osteoconductive matrix becomes a delivery system

for bioactive agents, requiring less chemotaxis and less migration of osteoblast progenitor cells to the graft site. The direct infusion of progenitor cells should lead to more rapid and consistent bone recovery. When an osteoconductive scaffold is seeded with bone morphogenetic proteins, for example, the composite graft may become both osteogenic and osteoinductive, providing a competitive alternative to autograft (Giannoudis et al. 2005; De Long et al. 2007). Such potential composite grafts are: bone marrow/synthetic composites, ultraporous β -TCP/BMA composite, osteoinductive growth factors and synthetic composites, BMP/polyglycolic acid polymer-composites and BMA/BMP/polyglycolic acid polymer-composite (Giannoudis et al. 2005).

Some of the commercially available composite grafts that are commonly used are Healos® (Orquest, Mountain View, CA), Collagraft® (Zimmer Corp, Warsaw, IN)

and Tricos® (Baxter BioSciences BioSurgery) ceramics (Biomatlante manufacturer, Vigneux de Bretagne, France).

Limited clinical data exist on the use of composite grafts in the treatment of periodontal defects (Sanders et al. 1983; Sottosanti 1993; Sottosanti 1995; Anson 1996; Anson 1998; Harris 2004; Harris 1998; Orsini et al. 2001; Maragos et al. 2002; Okuda et al. 2005; Orsini et al. 2008). Alloplasts can be mixed with autogenous grafts or allografts in the management of large structural defects (Zafropoulos et al. 2007). It was suggested that a mixture of autogenous bone and these materials should be used to overcome the lack of osseointegration of xenografts and alloplastic materials and to reduce the amount of bone resorption observed with pure autogenous grafts (Figs. 2.31 and 2.32). Thorwarth et al. (2006) demonstrated an accelerating

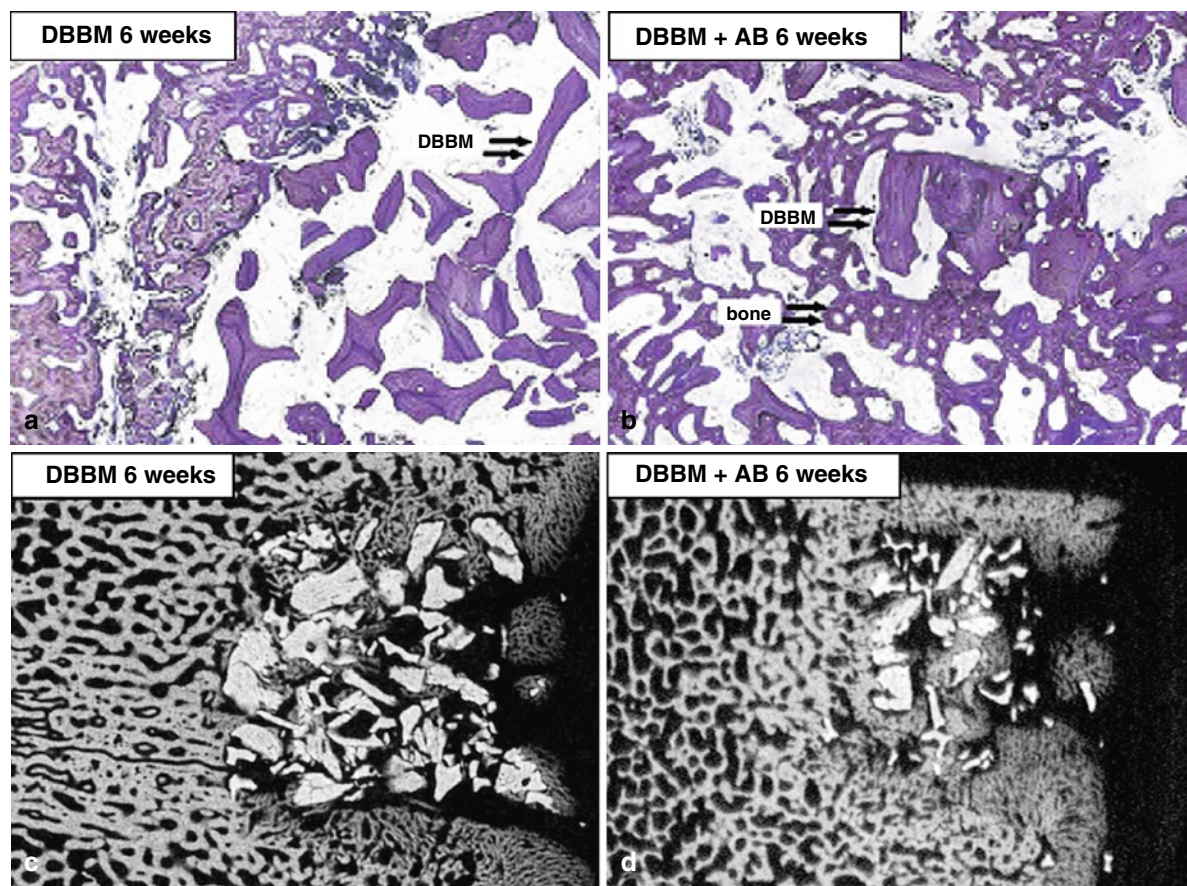


Fig. 2.31 De novo bone formation following application of autogenous bone to particulated anorganic bovine material in vivo. (a, b) Light microscopy at 6 weeks (toluidine blue O staining, magnification 35). (a) Group A = DBBM. (b) Group

B = DBBM 1.25% particulated AB. (c, d) Corresponding micro-radiographic images, enhanced de novo bone formation due to addition of particulated autogenous bone in group B (Thorwarth et al. 2006) (Reprinted with permission from Elsevier)

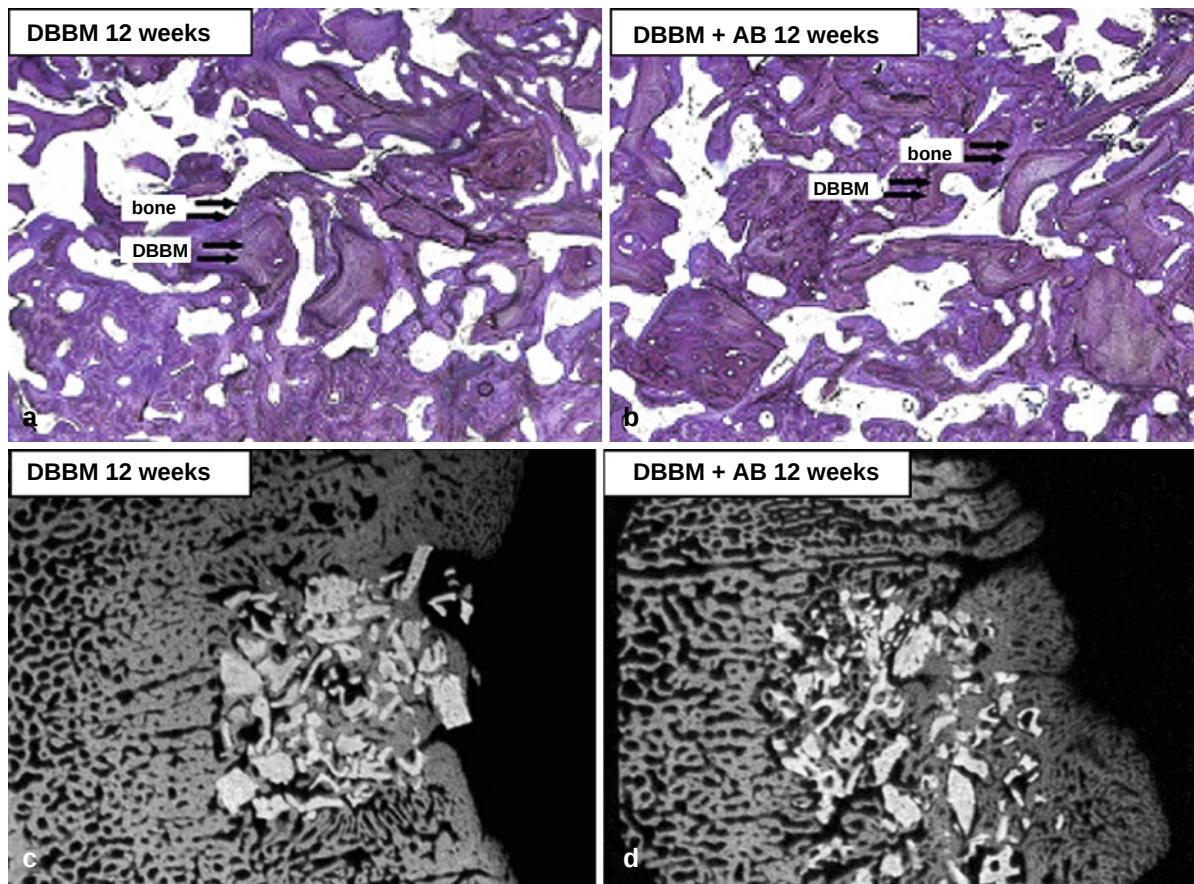


Fig. 2.32 De novo bone formation following application of autogenous bone to particulated anorganic bovine material in vivo. (a, b) Light microscopy at 12 weeks (toluidine blue O staining, magnification 35). (a) Group A = deproteinized bovine

bone matrix (DBBM). (b) Group B = DBBM + 25% particulated autogenous bone (AB). (c, d) Corresponding microradiographic images, adjustment of bone formation in both groups (Thorwarth et al. 2006) (Reprinted with permission from Elsevier)

effect of autogenous bone on bone formation in combination with deproteinized bovine bone matrix. This effect is likely caused by osteoinductive properties of cellular elements transplanted with the autogenous bone. It was also concluded that 25% autogenous bone was a sufficient volume to meet this accelerating effect on bone formation (Thorwarth et al. 2006). In deep intrabony defects treatment, at 12 months evaluation, the combined use of autogenous spongiosa with bovine-derived xenograft or with HA/ β -TCP led to significantly greater gain of clinical attachment and hard tissue formation compared to the use of autogenous spongiosa alone (Zafiropoulos et al. 2007).

In addition to these materials, research is continuing to modify the products with hopes of creating a graft that incorporates faster, resorbs and yields a bony union that resembles natural form and structure (Kuo et al. 2007).

2.6 Factors Impacting Treatment Outcome

2.6.1 Criteria for Evaluation of Graft Success for Periodontal Regeneration

For any graft material to be considered as a successful regenerative material, it should have clear histological, clinical and radiographic evidence of the following criteria (AlGhamdi et al. 2010a):

1. Biologic acceptability: the graft should not have any side effects or cause any unwanted tissue reaction.
2. Resorbability: the graft should resorb slowly and be replaced by the patient's own bone.

3. Regeneration: the graft should have evidence of regenerative ability with formation of new bone, cementum and periodontal ligament fibers.
4. Defect fill: the graft should have evidence of bone fill.
5. Stability: the outcome of the treatment should be stable at reevaluation visits.

2.6.2 Factors Influencing Graft Success

Several studies have investigated the possible sources of variability in the clinical outcomes of bone grafting procedures in periodontal surgery: (1) the patient, (2) the morphology of the defect, (3) the graft material, (4) the surgical procedure and (5) the healing period (Cortellini and Tonetti 2000; AlGhamdi et al. 2010a).

2.6.2.1 Patient Factors

The scientific literature clearly shows that plaque control (Cortellini et al. 1994), residual periodontal infection, tobacco smoking (Tonetti et al. 1995) and the patient's compliance (Wilson et al. 1984; Wilson et al. 1993b) are important prognostic factors in regenerative periodontal therapy. Other factors include conditions such as diabetes, hyperparathyroidism, thyrotoxicosis, osteomalacia, osteoporosis, Paget's disease and some medications may all affect the healing process (AlGhamdi et al. 2010a).

2.6.2.2 The Morphology of the Defect

Among the defect anatomy-associated factors, depth of the intrabony component of the defect and/or probing depth is consistently found to be relevant (Tonetti et al. 1996; Tonetti et al. 1998; Cortellini et al. 2001). The number of residual bony walls defining the defect seems to affect outcomes. Defects with two and three bony walls respond more favorably to treatment than do one-wall defects (Froum et al. 1976; Sepe et al. 1978). Also periodontal regeneration was more successful in deep-narrow defects than in shallow-wide defects (Dragoo and Sullivan 1973a; Froum et al. 1976; Mellonig 1984).

2.6.2.3 Selection of Graft Material

When bony reconstruction is presented to the surgeon, many choices must be weighed before the proper graft material is chosen (Kuo et al. 2007). Selection of graft material is guided by:

1. Biologic acceptability
2. Predictability
3. Resorbability
4. Clinical feasibility
5. Minimal operative hazards
6. Minimal postoperative sequelae
7. Patient acceptance (AlGhamdi et al. 2010a and references therein)

A range of 125–1,000 μm is acceptable with 250–750 μm most commonly available for particle size of grafts used in periodontal treatment. A minimal pore size of 100 μm is needed between particles to allow vascularization and bone formation. Particles less than 100 μm in size elicit a macrophage response and are rapidly resorbed with little or no new bone formation (Zaner and Yukna 1984; AlGhamdi et al. 2010a).

2.6.2.4 The Surgical Procedure

The surgical technique for the treatment of periodontal intrabony defects with bone replacement grafts is essentially the same regardless of the type of graft material being used. Incisions are designed to allow for primary closure of flaps to protect the graft site from infection and the graft material from displacement. Intrabony incisions are the common choice, with emphasis on preserving interdental tissue. Flaps are reflected full thickness to expose the underlying osseous defects and allow access for thorough debridement of the defects and meticulous root planning (Hanes, 2007). New surgical techniques have been developed to optimize primary closure as well as to minimize the surgical trauma in the reconstructive procedures of periodontal intraosseous defects. Recently, we proposed a minimally invasive procedure, the single-flap approach (SFA), specifically indicated when the defect extension is prevalent on the buccal or oral side. The basic principle of the SFA is the elevation of a flap to access the defect only on one side (buccal or oral), leaving the opposite side intact (Trombelli et al. 2009; Trombelli et al. 2010).

Once the defect has been debrided of soft tissue and the tooth root surfaces thoroughly planed to remove all deposits of dental plaque and calculus, the bone replacement graft material is packed into the defect to fill the defect to the level of the remaining alveolar bone (Hanes, 2007). Space maintenance is paramount to bone formation. If the graft material resorbs too rapidly, compared with the time required for bone formation, the site may fill with connective tissue rather than bone (AlGhamdi et al. 2010a). Therefore the space or contour and size of the augmentation should be maintained until the graft has formed enough bone to maintain the space itself (AlGhamdi et al. 2010a; Misch 1999; Polimeni et al. 2006). Absolute graft immobility is paramount to its union to the recipient bone. If pieces of bone graft are mobile, they cannot receive a blood supply, become encapsulated in fibrous tissue and often sequestrate (AlGhamdi et al. 2010a; Lin et al. 1990).

Flaps are closed and sutured for primary closure and complete coverage of the bone replacement graft (Hanes, 2007). Sutures should be removed in 7–10 days.

2.6.2.5 The Postsurgical Healing Period

Postsurgical care should include twice-daily rinsing with 0.12% chlorhexidine gluconate for 2 weeks and gentle toothbrushing starting 1 week after the surgery. Systemic antibiotics may be prescribed for 7–10 days after the surgical procedure. Patients should be seen at intervals of 1 week, 2 weeks and 4 weeks after surgery for supragingival plaque removal and then should be placed on a periodontal maintenance schedule at 3-month intervals (Hanes, 2007).

Adequate healing time must be provided to allow regeneration of the new bone volume. The amount of time required is variable and depends on local factors such as the number of remaining walls of bone, the amount of autogenous bone in the graft and the size of the defect. Larger grafts, less autogenous bone in the graft and fewer bony walls increase the amount of healing time (AlGhamdi et al. 2010a; Misch and Dietsh 1993; Misch 1999).

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Periodontal regeneration is defined as healing characterized by the formation of a new connective tissue attachment (new cementum with inserted collagen fibers) and new alveolar bone (Sculean et al. 1999a). Currently several treatment materials have been acknowledged as having fulfilled this criteria in human controlled clinical trials and human histology: intraoral autogenous bone, demineralized freeze-dried bone allograft (DFBA) alone and with bone morphogenetic proteins, guided tissue regeneration (GTR) and enamel matrix derivative (EMD) (Yukna and Mellonig 2000; Velasquez-Plata et al. 2002).

However, GTR techniques still present some unresolved problems: (1) a limit in the amount of regeneration achievable because of the membrane position; (2) membrane contamination in cases of exposure; and (3) delayed wound healing because of the degradation of resorbable membranes. Furthermore, the use of membranes requires a good degree of surgical skill and a long healing period (Parodi et al. 2000).

Enamel matrix derivative (Emdogain-Straumann, Basel, Switzerland) is the most widely studied commercially available bioactive agent. It is derived from the tooth pouches of unerupted porcine teeth (6-month-old piglets) and is composed of amelogenins and enzyme components. The biological rationale for the use of EMD is to recapitulate developmental mechanisms whereby enamel matrix proteins (EMPs) are proposed to play a critical role in stimulating cementogenesis (Esposito 2010).

3.1 EMD Formulation

The major fraction of the EMPs is composed of the amelogenins, a family of hydrophobic proteins that account for more than 90% of the organic constituent

of the enamel matrix. The second largest component of the EMPs is the enamelines. Since the enamelines were found to contain serum proteins, the more general term “non-amelogenin” is now commonly used to describe this high-molecular-weight fraction. It includes proline-rich enamelin, tuftelin and tuft proteins (Hammarström et al. 1997; Venezia et al. 2004 with references therein).

An aqueous solution of propylene glycol alginate (PGA), a non-setting alginate, was found to be a suitable vehicle for Emdogain, permitting application via a syringe due to its shear-thinning properties. Propylene glycol alginate is viscous at low pH values and cool temperatures but when the acidity is neutralized and the temperature increased, e.g., by tissue fluids, the viscosity decreases dramatically and Emdogain is released, precipitating on the exposed surfaces of the surgical area, including the root surface. Amelogenins form typical supramolecular assemblies under physiological conditions facilitating periodontal ligament (PDL) cell attachment and growth (Gestrelus et al. 2000). It has been revealed that the PGA vehicle solution of the EMD has significant antimicrobial effects on periodontal pathogens (Arweiler et al. 2002; Sculean et al. 2001a; Spahr et al. 2002).

The first marketed EMD product was supplied in a lyophilized form and was dissolved in an aqueous solution of PGA immediately prior to use. Because mixing EMD with PGA needs extra assistance and time, a new ready-to-use product, Emdogain® Gel (Biora AB, Malmö, Sweden), was developed. It is a premixed formulation of EMD, where the protein has been stabilized by heat treatment prior to being mixed with the vehicle. Both formulations contain 30 mg EMD protein/mL PGA gel, with a viscosity of about 2.5 PAS (and shear-thinning rheology) (Venezia et al. 2004). Bratthall et al. (2001) compared the clinical and

radiographical outcome of a ready-to-use Emdogain-gel (test) with the marketed Emdogain (control). Eighty-eight subjects with bilateral infrabony defects ≥ 4 mm deep and ≥ 2 mm wide according to radiographs were selected and enrolled in a blinded randomized controlled multicenter study. At baseline, the mean probing depth (PD) was 7.8 mm for the test and 7.8 mm for the control sites. Eight months postoperatively the mean test PPD was reduced to 4.4 mm and the mean control PPD to 4.5 mm. The corresponding figures at 16 months were 4.1 and 4.2 mm, respectively. There was no statistically significant difference between (paired) test and control sites at baseline, 8 and 16 months postoperatively. The clinical attachment level (CAL) measurements demonstrated a gain of attachment of 2.3 mm for the test and 2.5 mm for the control sites at 8 months. At 16 months the gain of attachment increased to 2.7 and 2.9 mm, respectively. There was no statistical difference between (paired) test and control sites at baseline, 8 and 16 months postoperatively (Bratthall et al. 2001).

3.2 Clinical Safety of EMD

Since EMD is a porcine-derived material, it might have the potential of stimulating immune reactions in humans (Esposito et al. 2009). However, EMDs are quite similar among mammalian species (Brookes et al. 1995) thus are less likely to be antigenic (Esposito et al. 2009). Multiple exposures to EMD during periodontal therapy have been shown to be safe for the patient (Froum et al. 2004; Heard et al. 2000; Zetterström et al. 1997). Few minor postoperative complications (6%) were reported in clinical trials at EMD-treated sites (Sanz et al. 2004), while only one report (St George et al. 2006) described in two cases of inflammatory external root resorption in association with EMD treatment dictating tooth extraction.

3.3 Biomimicry

Biomimicry has been defined as a new science that studies nature's models and then imitates or takes inspiration from these designs and processes to solve human

problems. The treatment of periodontal defects with Emdogain is a good example of biomimicry. The use of an insoluble matrix that initiates a series of cellular events may be especially favorable since the cells involved then provide the growth factors and tissue components necessary for regeneration (Gestrelius et al. 2000).

3.4 Mechanisms Underlying the Supportive Effects of EMD

A technique or a material must fulfill the following criteria in order to be classified as "regeneration-promoting" (AAP 1996; Sculean et al. 2007c):

- In vitro studies, which confirm the action mechanism
- Controlled histological animal studies, which demonstrate formation of new root cementum, periodontal ligament (PDL) and alveolar bone
- Human biopsies, which show formation of root cementum, PDL and alveolar bone on a plaque-infected root surface
- Controlled clinical studies, which prove a gain of clinical attachment and radiological new bone formation

In the following overview, the existing evidence regarding the in vitro and in vivo studies investigating the mechanisms underlying the supportive effects of EMD is provided.

3.4.1 In Vitro and In Vivo Experiments

Enamel matrix proteins (EMPs) have attracted considerable attention since their launch as medical devices. There is a vast amount of biological information available on functions of EMPs that go beyond both the regulation of enamel mineral crystal growth and the original idea of a function in cementoblast differentiation, which was actually the basis for commercialization. It is now evident that EMPs affect many different cell types and that not all the results are consistent. It is also clear that the results cannot always be consistent. There are several reasons for this, including the use of (1) different types of EMPs, (2) different concentrations of EMPs, (3) different observation periods, (4) different

cell types, (5) different states of cell differentiation, (6) different experimental in vitro systems or conditions and (7) different local in vivo environments. Nevertheless, there is a large body of information available that provides a biological rationale for the use of EMPs for periodontal regeneration (as reviewed by Bosshardt 2008) (Table 3.1).

Overall, the available data suggest the following:

1. *Cell attachment, spreading and chemotaxis:* In most studies, EMPs caused an increase in cell attachment of epithelial cells, gingival fibroblasts and PDL fibroblasts. Regarding differences in the rate and extent of cell attachment between gingival and PDL fibroblasts, inconsistent observations were made. A promotion of adhesion of osteogenic cells also does occur, but appears to be dependent on the cell differentiation/maturation state. Cell–matrix adhesion appears to be mediated, at least in part, by integrins. EMD also has a chemotactic effect on endothelial cells (Bosshardt 2008).
2. *Cell proliferation and survival:* Most information is available on the effects of EMPs on cell proliferation. EMPs favor cell proliferation of PDL fibroblasts over gingival fibroblasts and over epithelial cells. Epithelial cells appear to respond the least to EMPs by cell proliferation. However, the effect of EMPs on epithelial cells appears to be cytostatic, but not cytotoxic. The influence of EMPs on cell proliferation of osteogenic cells including various progenitors appears to decrease with increasing cell differentiation/maturation state. Accelerated wound-fill rates in vitro using PDL fibroblasts, gingival fibroblasts and osteoblast-like cells appear to be due to enhanced cell migration and proliferation. EMPs stimulate the outgrowth of new blood vessels and increase the number of endothelial cells (Bosshardt 2008).
3. *Expression of molecules involved in the regulation of bone remodeling:* EMPs have an influence on this system by modulating the expression of OPG and RANKL. It was suggested that EMPs modulate the RANK-RANKL-OPG system most likely toward bone apposition. Furthermore, it has also to be taken into consideration that some of the growth factors and cytokines that are upregulated by EMPs directly upregulate OPG and downregulate RANKL production. Thus, EMPs appear to be indirectly involved in the regulation of bone remodeling (Bosshardt 2008).

Several studies have also suggested that Emdogain has also an antibacterial effect on ex vivo dental plaque vitality (Sculean et al. 2001a), on in vitro growth of gram-negative periodontal pathogens like *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia* (Spahr et al. 2002; Newman et al. 2003; Walter et al. 2006) and on established supragingival plaque in periodontally healthy dental students (Arweiler et al. 2002). The results also suggest that PGA contributed most to this activity (Sculean et al. 2001a; Arweiler et al. 2002; Newman et al. 2003; Walter et al. 2006).

3.4.2 Animal Experiments

Animal data (Sakalliglu et al. 2004; Onodera et al. 2005; Sculean et al. 2000b, 2000c, 2000d; Cochran et al. 2003; Donos et al. 2003; Hamamoto et al. 2002; Hammarström et al. 1997; Nemcowsky et al. 2006; Regazzini et al. 2004; Sallum et al. 2003, 2004) (Table 3.2) indicate that EMD is present on the treated root surfaces for a period of at least 4 weeks and predictably it promotes formation of cementum, periodontal ligament and bone in fenestration, recession, intrabony and mandibular class II furcation defects (Sculean et al. 1999b, 2007c).

3.4.3 Human Histological Studies

Based on the available evidence from human histological studies (Bosshardt et al. 2005, 2006; Carnio et al. 2002; Heijl 1997; Mazjoub et al. 2005; McGuire and Cochran 2003; Mellonig et al. 2009; Mellonig 1999; Rasperini et al. 2000; Sculean et al. 1999b, 2002a, 2002c, 2003a, 2003c, 2003d, 2008a, 2008c; St George et al. 2006; Yukna and Mellonig 2000), summarized in Table 3.3, it may be concluded that the application of EMD in conjunction with periodontal surgery may promote formation of new cementum, PDL and bone in intrabony and recession defects (Figs. 3.1–3.3). Moreover, when applied during periodontal surgery EMD can be detected on the root surfaces for a period of at least 4 weeks (Figs. 3.4 and 3.5). Based on current knowledge, there are no histological data from human material evaluating the regenerative potential of EMD in furcation defects (Sculean et al. 2007c).

Table 3.1 Effects of EMD on various cell types in vitro

Process/ molecules	Epithelial cells	Gingival fibroblasts	PDL or follicle cells	Cementoblasts	Osteoblasts	Pre- osteoblasts	BMSC or mesenchymal progenitors	Chondrogenic cells	Endothelial cells
<i>Cell attachment, cell spreading, chemotaxis</i>									
	+, +	+, +	-, +, +, +, -, -		+, +				+, +
<i>Cell proliferation</i>									
	-, -, -, -, +	+, +, +, +, +, +, +	+, +, +, +, +, -, - +, +, -, +, +, -, +, -, +, -, +, +, +, +	+	+, +, +, -, +, +, +, +, -, +, +, -, +	+, +, +	-, +, -, +	+, +	-, +, +
<i>Growth factors, cytokines</i>									
TGF- β 1		+	+, +, +, +, +		+, +, +			-	
BMP-2			+						
BMP-7			+						
PDGF-AB	+		+						
CTGF					+				
FGF-2					+				
IGF-1			+, +, +		-	+			
VEGF			+						
TNF- α									
IL-6			+		+				
IL-8									
PGE ₂								+	
COX2			+		+				
<i>Total protein synthesis, extracellular matrix molecules</i>									
Total protein		+	+, -, +						
Collagen type I			-		+, +, +	+	-, -		
Collagen type II								+	
Collagen type X							+	+	
Proteoglycans	+	+	+					+	

OPN	+	+	+,+,+	+	+,+	+	
BSP	-	-	+,+,+,+	+	+,+	+	
OC			-,+,+	-	-,+,+,+	-,+	-,+, -
CAP			+				
CP-23			+				
MMPs					+, -		+
<i>Mineralization</i>							
In vitro mineral- ized nodule formation		-	+, -, +, -, +, +		+, +, +, -	+, -	+
ALP		+, -	+, -, +, -, +, +		+, +, -, +, +, +, -	+	-, +
<i>Bone remodeling</i>							
RANKL			-		-		
OPG			-, +		+	+	
Intracellular signaling molecules, transcription factors							
Cbfa1/Runx2			-			+	+, +
Osx							
Sox9						+	+
Zfp60							+
AJ18							+
cAMP		+, -	+				

Source: Bosshardt (2008). Reprinted with permission from John Wiley & Sons, Inc.

Every single + or - indicates one single study or single cell line, if more than one cell line was used in the same study

+ positive effect, - negative or no measurable effect, empty cells not determined or not applicable, BMSC bone marrow stromal cells

Table 3.2 Animal studies on the effects of EMD on formation of cementum, periodontal ligament and bone in fenestration, recession, suprabony, intrabony and furcation defects

Reference	Design	Animal model	No. animals	Defect type	Tooth	Treatment (n)	Study period	Outcome
Cochran et al. (2003)	Split-mouth	Monkeys	5	Intrabony 4 one-walled defects	Canine	1. SRP + EDTA + EMD (20)	5 months	Qualitatively, new cementum, periodontal ligament with Sharpey's fibers, and new bone tissue were observed. In general, enamel matrix protein treatment resulted in greater tissue formation than controls. In many instances, dramatic tissue formation occurred far coronal to the base of the defects. The height of new cementum in the 1-mm-wide defects was 2.85 mm compared with the 2.33 mm for the controls. In the 2-mm-wide lesions, the EMD-treated sites had a new cementum height 3.57 mm compared to 2.10 mm for the controls. In 4-mm-wide lesions, the EMD-treated sites had a new cementum height of 2.38 mm, while controls had 1.55 mm of new cementum. In the 6-mm-wide defects, the amount of new cementum in EMD and control sites was similar, with 2.71 and 2.75 mm, respectively. When evaluating the combined 1 and 2 mm defects, the height of new cementum with enamel matrix protein treatment was 45% greater than the control, with 31% greater new bone height versus the control. In the combined wider defects (4 and 6 mm), new tissue height was more similar between enamel matrix protein-treated defects and control defects. In addition, horizontal bone fill occurred in defects that were initially 4 or 6 mm wide. The resultant width of the periodontal ligament was similar in all defects regardless of the original defect width
					Premolar Molar	2. SRP + EDTA (control) (20)		

Donos et al. (2003)	Split-mouth	Monkeys	3	Degree III furcation defects	Molar	1. SRP + 24% EDTA gel 2 min + GTR (3) 2. SRP + 24% EDTA gel 2 min + EMD (3) 3. SRP + 24% EDTA gel 2 min + EMD + GTR (3) 4. Coronally displaced flap (control) (3)	5 months	The histological analysis revealed that with GTR or combined EMD and GTR treatment, new attachment formation (new cementum with inserting collagen fibers) had occurred on almost the entire circumference of the furcation and new bone was almost filling the defect in the situations where the membrane was not exposed. The sites treated only with EMD exhibited new attachment and new bone formation to a varying extent. In one specimen, it covered 87.4% of the circumference of the defect, while it was 42.4% and 46.1% in the remaining two specimens. In the lower portion of the defects, the new cementum was of an acellular type, whereas in the coronal portion, a mixed cellular and acellular type of cementum was observed. The fomix of the furcation did not present new cementum in any of the specimens. The newly formed bone amounted from 51.5% to 92.4% of the height of the defect. All control furcations were open with epithelialized inflamed connective tissue in the lower portion of the defect, and new cementum with inserting collagen fibers and new bone was limited to the level of the notch
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Table 3.2 (continued)

Reference	Design	Animal model	No. animals	Defect type	Tooth	Treatment (n)	Study period	Outcome
Hammarström et al. (1997)	Parallel	Monkeys	29	Buccal dehiscence model	From canine to the first molar	1. Enamel matrix (16) 2. Acidic extract (12) 3. EDTA/buffer extract (6) 4. EMD (30) 5. Control (sham operation) (30)	8 weeks	Application of homogenized enamel matrix or an acidic extract of the matrix containing the hydrophobic low-molecular-weight proteins, anelogenins, resulted in an almost complete regeneration of acellular cementum (60–80%), firmly attached to the dentin and with collagenous fibers extending over to newly formed alveolar bone. After application of fractions obtained by neutral EDTA extraction containing the acidic, high-molecular-weight proteins of the enamel matrix, very little new cementum was formed and hardly any new bone. The results of the controls in which no test substance was applied before the repositioning of the flap, were very similar to those obtained with the EDTA extracted material
Nemcowsky et al. (2006)	Parallel	Wistar rats	20	Combined supra-infrabony defects	Molar	1. SRP + 24% EDTA gel 2 min 2. SRP + 24% EDTA gel 2 min + EMD	12 weeks	New cementum was distinguished from the native cementum remaining on the root surface in organization and cellular contents. New cementum could be observed only in the EMD group, mainly localized on the remaining old cementum in the apical portion of the defect (i.e., in the infrabony part). The length of ankylosis between bone and tooth was more than six times smaller in the EMD group, but due to the large within-groups variation, this difference was not statistically significant. The extent of supracrestal connective tissue was similar in both groups. However, in the EMD group, this tissue was more organized. Area and length of new bone formation did not differ between the two groups. A significantly higher area of new cementum was found in the EMD group ($P = 0.02$)

Onodera et al. (2005)	Split-mouth	Dogs	6	Intrabony defects	Premolar	1. Control (GTR alone) (6) 2. Exptl. group treated (GTR + EMD) (6)	8 weeks	After 2 and 4 weeks, new bone and new cementum formation in the experimental group were significantly greater than those in the control group (2 weeks: new cementum: 19.7% vs. 9.3%, new bone: 24.9% vs. 10%; 4 weeks: new cementum: 84% vs. 50.2%, new bone: 77.6% vs. 55.2%) ($P < 0.05$). However, after 8 weeks, no statistical difference was found between the two groups in new bone or cementum formation. The study results suggest that a maturation of periodontal ligament cells might contribute, during the early stage of periodontal healing, to stimulate a proliferation of periodontal ligament cells
Regazzini et al. (2004)	Split-mouth	Dogs	4	Class II furcation	Molar	1. Control 2. GTR + EMD 3. EMD	8 weeks	Healing in control group was characterized by a long junctional epithelium and discrete bone formation; GTR + EMD group showed reduced bone formation; and EMD group showed significant bone regeneration (area of new bone = $67.36 \pm 3.93\%$; distance from furcation roof to bone crest = 0.57 ± 0.15 mm). The EMD led to significant regeneration of the furcation lesions, and the association with membranes was detrimental

(continued)

Table 3.2 (continued)

Reference	Design	Animal model	No. animals	Defect type	Tooth	Treatment (n)	Study period	Outcome
Sakallıoglu et al. (2004)	Split-mouth	Dogs	4	Suprabony defect	Premolar	1. SRP + 36% orthophosphoric acid for 15 s (7) 2. SRP + 36% orthophosphoric acid for 15 s + EMD (7)	28 days	Connective tissue attachment and proliferation rate was significantly higher ($P < 0.01$) in the test roots than the control roots at day 7 (3.29 ± 0.3 mm vs. 1.02 ± 0.32 mm). When this rate was quantitated and evaluated from day 7 to day 28, an increasing degree of connective tissue attachment and a decreasing degree of epithelial attachment were noted in the test group unlike the control samples. Orientation of the collagen fibers of supra-alveolar connective tissue and PDL fibers were established at day 21 and could be observed at day 28 in the test and control group. Formation of new bone initiated at day 14 in both groups and osteoblastic proliferation and bone maturation leading to lamellar bone formation was observed to be more activated within the test samples than within the control ones. The most significant change in the hard tissues in addition to bone maturation at day 21 was the initiation of cementum by progenitor cells in the test and control roots. At day 28, the regenerated cementum in all of the control group sections was apparently cellular. A firm attachment of acellular cementum to the root dentin with functional organization of its collagen fibers was noted. Attachment of new acellular cementum to the dentin surface and organization of its collagen fibers was not significantly different than new cellular cementum formed in the test group. When the new cementum formation in test (2.01 ± 0.14 mm) and control (1.38 ± 0.26 mm) groups at day 28 were compared by means of length, the difference was found to be statistically

Sallum et al. (2003)	Split-mouth	Dogs	5	Recession	Upper cuspid	3 months	The gingival recession was -0.1 ± 0.2 mm for the EMD-Group and -0.8 ± 1.3 mm for the CPF-Group ($P=0.17$). The extension of the epithelium was 1.2 ± 1.0 mm for the EMD-Group and 1.3 ± 0.7 mm for the CPF-Group ($P=0.89$). The new connective tissue attachment was 4.8 ± 0.7 in the EMD-Group and 4.0 ± 1.4 in the CPF-Group ($P=0.22$). The new bone was 0.1 ± 1.8 mm and -0.5 ± 1.4 mm in the EMD-Group and CPF-Group, respectively ($P=0.50$). Histologically, the defect coverage observed was 98.2% for the EMD-Group and 85.8% for the CPF-Group
					1. Coronally positioned flaps associated + EMD (EMD-Group) 2. Coronally positioned flaps alone (CPF-Group)		

(continued)

Table 3.2 (continued)

Reference	Design	Animal model	No. animals	Defect type	Tooth	Treatment (n)	Study period	Outcome
Sallum et al. 2004	Split-mouth	Dogs	7	Buccal osseous dehiscences	Premolar	1. Open flap debridement (OFD)(7) 2. GTR (7) 3. Root instrumentation + EDTA 24% (2 min) + EMD (7) 4. Root instrumentation + EDTA 24% + EMD + GTR (7)	4 months	The predominant healing pattern for OFD was the development of a long junctional epithelium on the coronal half of the defect. The mean length of the epithelium observed in EMD+GTR (0.50 mm) was approximately half of the length in the OFD (1.11 mm), while the other two treatments showed an intermediate result (0.78 mm for both groups). The new cementum formation was superior for sites treated with EMD (EMD = 3.72 mm and EMD + GTR = 3.78 mm) compared to the OFD (2.40 mm) ($P < 0.05$). GTR showed 3.01 mm of new cementum formation, with no significant differences compared to the other groups ($P > 0.05$). Regarding new bone formation, no statistically significant differences were observed among the treatments; however, the magnitude of the values indicated a superior bone formation for EMD (2.01 mm) followed by EMD + GTR (1.40 mm) when compared to OFD (1.14 mm) and GTR (0.91 mm)($P > 0.05$)

Sculean et al. (2000b)	Split-mouth	Monkeys	3	Fenestration	Canine	1. 24% EDTA gel for 2 min + GTR (4) 2. 24% EDTA gel for 2 min + EMD (4) 3. Control (24% EDTA gel for 2 min + coronally repositioned flaps) (4)	5 months	In all defects treated with GTR, new attachment and new alveolar bone were consistently reformed. The amount of newly formed cementum varied from 3.45 to 3.91 mm (100% of the original defect size), and the amount of new bone varied from 3.41 to 3.90 mm (100% of the original defect size). In all four defects, the healing occurred in a complete closure of the defect. In the defects treated with EMD, new attachment and new bone formation occurred to a varying extent. The amount of newly formed cementum varied from 1.58 to 3.85 mm (30–100% of the original defect size), and the amount of new bone varied from 0.0 to 3.85 mm (0–100% of the original defect size). Complete closure of the defects occurred in two out of the four defects. In the controls, the amount of new cementum varied from 1.12 to 3.75 mm (20–100% of the original defect size) and the amount of new bone varied from 0.99 to 3.75 mm (15–100% of the original defect size). Complete closure of the defects occurred in two out of the four defects. The observation that after treatment with EMD the amount of the newly formed cementum and bone was not superior to that formed after flap surgery alone, may be explained with a lack of space due to an eventual collapse of the mucoperiosteal flap into the wound
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Table 3.2 (continued)

Reference	Design	Animal model	No. animals	Defect type	Tooth	Treatment (n)	Study period	Outcome
Sculean et al. (2000c)	Split-mouth	Monkeys	3	Intrabony defects	Incisors Premolars	1. GTR 2. EMD 3. EMD+ GTR 4. Controls (coronally repositioned flaps)	5 months	In the control specimens, the healing was characterized by a long junctional epithelium and limited periodontal regeneration (i.e., new periodontal ligament, new cementum with inserting connective tissue fibers and new bone) in the bottom of the defect. The new attachment formed after treatment with GTR consisted of a predominantly cellular type of cementum with inserting collagen fibers oriented perpendicularly to the tooth surface. The amount of newly formed attachment varied from 2.95 to 3.61 mm and the amount of new bone varied from 2.25 to 3.45 mm. In the defects treated with EMD, periodontal regeneration occurred to a varying extent. In the lower portions of the defects, the newly formed cementum was of an acellular type, whereas in the more coronal portion of the defects, a mixed cellular and acellular cementum was often observed. Collagen fibers were seen inserting into the newly formed cementum oriented perpendicularly to the tooth surface. The amount of newly formed attachment varied from 0.40 to 3.85 mm and the amount of new bone varied from 0.58 to 2.88 mm. In the defects treated with the combined therapy, the healing resulted consistently in new attachment and new bone formation when the membranes were not exfoliated or exposed. At the sites without membrane exposure the amount of new attachment varied from 2.31 to 3.68 mm and the amount of new bone varied from 2.77 to 3.67 mm

CT connective tissue, GTR guided tissue regeneration, L/JE long junctional epithelium, NC new cementum, NB new bone

Table 3.3 Human histological studies on the effects of EMD on formation of cementum, periodontal ligament and bone in fenestration, recession and intrabony defects

Reference	Defect type/n subjects	Treatment evaluated	Study length	Analysis	Outcome
Bosshardt et al. (2005)	12 advanced intrabony periodontal defects (1, 2, 3-wall)/10 patients (5 females and 5 males; mean age of 50 years)	Full-thickness mucoperiosteal flaps + 24% EDTA gel 2 min + EMD	6 weeks	Light and transmission electron microscopy	New tissue formation on the root was observed in the notch and on both scaled and unscaled root surfaces distant of the notch area in six defects. The newly formed tissue in the root notch was thick, had an irregular surface contour, was devoid of extrinsic fibers, contained embedded cells and the cells on the matrix surface were very large. Although the morphological appearance of the interface between the treated root surfaces and the newly formed mineralized tissues varied, a gap or split was consistently observed in the coronal-most portion of the instrumented area. The space of the gap appeared empty or was filled with an organic material resembling scattered or colonies of bacteria. The matrix of the newly formed tissue on the root was collagenous. The collagen fibrils were randomly oriented and loosely packed and a distinct mineralization front was not discernible. However, large, electrondense matrix patches were scattered in and adjacent to the new tissue. The cells that lined the new tissue were very large and possessed abundant cisternae of rough endoplasmic reticulum and a prominent Golgi complex. Embedded cells were enclosed in a lacuna and a canalicular system filled with cytoplasmic processes connecting neighboring lacunae was observed. In most cases, the coronally located gap between the new tissue and the instrumented root surface was filled with scattered or colonies of bacteria

(continued)

Table 3.3 (continued)

Reference	Defect type/ <i>n</i> subjects	Treatment evaluated	Study length	Analysis	Outcome
Bosshardt et al. (2006)	Same as previous	Same as previous	Same as previous	Light and transmission electron microscopy IHC	The newly formed tissues on the root were thick and contained embedded cells. Small mineralization foci were regularly seen and large organic matrix patches were occasionally seen, but a distinct mineralization front was lacking. Within the mineralized portions of the alveolar bone, labeling for BSP and OPN was preferentially associated with small, isolated organic patches displaying a reticular texture. The mineralized compartment of the cellular intrinsic fiber cementum (CIFC) matrix was characterized by electron-dense matrix patches that varied in size and consisted of a granular organic matrix that labeled for OPN and BSP. Mineralization foci were present adjacent to the clearly demarcated mineralization front. Labeling for OPN and BSP was associated with these mineralization foci and with the electron-dense, interfibrillar organic matrix. Within the mineralized compartments of the newly formed tissues on the root of EMD-treated teeth, labeling for BSP was mainly associated with small, isolated, organic material exhibiting a reticular texture. Labeling for OPN, in association with the large, irregularly shaped, electron-dense matrix patches, was consistent. Strong labeling of these matrix structures was consistently observed for BSP

Camio et al. (2002)	4 maxillary teeth (3 canines and 1 first premolar) that presented mucogingival problems and Miller's class II and III gingival recession in 2 female subjects (2 in each patient)	Root coverage procedures performed with a combination of a connective tissue (CT) graft and an enamel matrix derivative (EMD) preparation	6 and 12 months	H	All treated teeth presented with clinical signs of successful root coverage. Histological observations of the 4 specimens revealed similar findings. The junctional epithelium was ≤ 2 mm long. Apical to the junctional epithelium, dense CT fibers were found in close proximity to the root surface, but in general, no insertion of these fibers into the root was observed. In one 6-month specimen, formation of new cementum and new bone was observed in the most apical end of the grafted area. The results of this study suggest that a combination of CT grafts and EMD results mainly in an adhesion between the CT and root surface. Some periodontal regeneration may occur in some regions. The development of a long junctional epithelium was not observed with this combined therapy for the treatment of gingival recession
Heijl (1997)	Buccal dehiscence defect in a mandibular incisor in one male subject	Full-thickness (mucoperiosteal) buccal flap + 37% ortho-phosphoric acid for 15 s + EMD	4 months	H	Microscopic examination showed the formation of anacellular extrinsic fiber cementum, which was firmly attached to the underlying instrumented dentin surface. The new cementum was thin and contained collagen fibers, which had a direction that was at right angles to the long axis of the root and also extended into an associated periodontal ligament. An alveolar bone attached to the ligament was also present. The morphometric measurements showed that there was an apical recession of the soft gingival tissues leaving an exposed root surface amounting to 15% of the original defect as measured from the coronally placed notch to the apical bevel. The new cementum layer covered 73% of the original defect and the junctional epithelium had proliferated slightly in an apical direction to cover 12% of the defect. Alveolar bone gain was 65% of the presurgical bone height

(continued)

Table 3.3 (continued)

Reference	Defect type/ <i>n</i> subjects	Treatment evaluated	Study length	Analysis	Outcome
Mazjoub et al. (2005)	One deep intrabony defect at the distal root of a mandibular first molar in one 46-year-old woman	Full-thickness (mucoperiosteal) buccal flap + 24% EDTA 2 min + EMD	9 months	H	Histologic analysis demonstrated two different patterns of healing along the proximal and furcal surfaces. Regeneration with new cellular cementum, bone and periodontal ligament with functional fiber orientation was observed on the distal aspect of the root, whereas the furcal surface healed through ankylosis
McGuire and Cochran (2003)	4 hopeless teeth with Miller class IV recession in one 29-year nonsmoking patient	1. Root coverage with subepithelial connective tissue graft	6 months	H	Histological evaluation of the subepithelial connective tissue graft revealed a long junctional epithelium ending at the coronal extend of the notch representing the preoperative gingival margin. Below the apical extent of the long junctional epithelium, connective tissue was found adjacent to the dentin in the notch. No histological evidence of cementum, bone or periodontal ligament and, therefore, regeneration was noted. In addition, there appeared to be some resorption of the dentin adjacent to the graft. Histological evaluation of the coronally advanced flap with EMD illustrated all the tissues necessary for regeneration: the presence of new cementum, organizing PDL fibers and islands of condensing bone at a constant distance from the root surface
		2. Root coverage with coronally advanced flap with enamel matrix derivative (EMD)			These histologic sections strongly suggest that enamel matrix derivative works in a biomimetic fashion by mimicking the natural process of tooth development

Melloni et al. (2009)	4 sites (upper right central incisor with horizontal bone loss except for a slight <1.0-mm angular defect; left maxillary canine with an angular bony defect of 4.0 mm; right maxillary central incisor with an angular bone defect of 3.0 mm and a PD of 13 mm; and a lower left lateral incisor with a CAL of 7.0 mm and a PD of 7.0 mm) in 4 patients with severe chronic periodontitis (PD and CAL >7.0 mm) and scheduled for complete dentures (one female and three males; mean age: 58 years; range: 37–77 years)	Subgingival scaling and root planing with a diamond-tipped ultrasonic instrument in an aggressive manner as possible without anesthesia + EMD 30 mg/ml, was inserted into the pocket to the base of the lesion and was allowed to exude from the pocket. No EDTA or other root surface modification was used	6 months	H	Probing depth reduction and clinical attachment level gain were obtained in three-fourths of the specimens. Three of the four specimens analyzed histologically demonstrated new cementum, bone, periodontal ligament and connective tissue attachment coronal to the notch. In one specimen, the gingival margin had receded below the notch. The results were unexpected and the authors considered that it may represent an aberration. Several possible explanations for the results in this study were presented: (1) The histologic reference notch was placed in a non-contaminated zone on the root; (2) there was a lack of controls, but this study was meant to be a case report of unusual observations; (3) there was a bias toward favorable results; (4) there was no limit on the time for scaling and root planing; (5) a 2-week recall would help to control bacterial plaque and, therefore, promoted a better outcome
Rasperini et al. (2000)	A mandibular canine with 6 mm of recession as measured from the cemento-enamel junction to the gingival margin, minimal pocketing and no keratinized gingival in one patient	Subepithelial connective tissue graft (SCTG) + EMD	6 months	H	The tooth demonstrated a 2-mm gain of attachment and a 3-mm gain in keratinized tissue. The histologic study evidenced the migration of the junctional epithelium 1.2 mm apical to the sulcus. New cementum, evidence of newly formed woven bone and connective tissue fibers anchored in the new cementum were evident

(continued)

Table 3.3 (continued)

Reference	Defect type/ <i>n</i> subjects	Treatment evaluated	Study length	Analysis	Outcome
Sculean et al. (1999c)	14 advanced intrabony defect around teeth scheduled for extraction for periodontal and prosthetic reasons in 14 patients with adult periodontitis	1. Full-thickness (mucoperiosteal) buccal flap + 24% EDTA 2 min + EMD 2. Full-thickness (mucoperiosteal) buccal flap + GTR (bioresorbable membrane resolut)	6 months	H	At baseline the mean PD in the EMD group was 11.3 ± 1.8 mm and the mean 12.1 ± 2.0 mm, whereas in the GTR group the mean PPD was 11.4 ± 2.2 mm and the mean CAL 13.3 ± 2.3 mm. The clinical results revealed at 6 months in the EMD group a mean PPD of 5.6 ± 1.3 mm and a mean CAL of 9.1 ± 1.5 mm. In the GTR group, the mean PD was 5.6 ± 1.3 mm and the mean CAL 10.1 ± 1.5 mm. The histological analysis showed in the EMD group a mean 2.6 ± 1.0 mm of new attachment (i.e., new cementum with inserting collagen fibers) and a mean 0.9 ± 1.0 mm of new bone. In this group, the formation of new attachment was not always followed by bone regeneration. In the GTR group, the mean new attachment was 2.4 ± 1.0 mm and the mean new bone 2.1 ± 1.0 mm. In every case treated with GTR, the formation of new attachment was followed by a varying amount of new bone. After both types of regenerative treatment, the newly formed cementum displayed a predominantly cellular character
Sculean et al. (2000a)	2 deep intrabony defects adjacent to teeth scheduled for extraction in 2 patients with marginal periodontitis	Full-thickness (mucoperiosteal) buccal flap + 24% EDTA 2 min + EMD	6 months	H	Following treatment, newly formed cementum with inserting collagen fibers was found in both specimens. In one case, the new attachment formation was also accompanied by bone neoformation. The results of this human histologic study indicate that Emdogain possesses the potential to stimulate new connective tissue attachment formation in human intrabony defects

Sculean et al. (2002c)	16 advanced intrabony defects around teeth scheduled for extraction for periodontal and/or prosthodontic reasons in 16 patients (10 females and 6 males with a mean age of 35 ± 5.2 years) suffering from chronic periodontitis	1. Full-thickness mucoperiosteal flaps + 24% EDTA gel for 2 min + EMD 2. Full-thickness mucoperiosteal flaps	4 weeks	IHC	The results demonstrate that EMD is present on treated root surfaces of periodontitis patients during a period of 4 weeks after application and indicates that these proteins are stable and sufficiently long lived to facilitate recruitment and recolonization of PDL cells onto the root surface
Sculean et al. (2003c)	8 advanced intrabony periodontal defects around teeth scheduled for extraction for periodontal or prosthodontic reasons in 8 patients suffering from chronic periodontitis	Full-thickness mucoperiosteal flaps + 24% EDTA gel for 2 min + EMD	6 months	IHC	In all specimens, the healing resulted to a varying extent in formation of cementum, periodontal ligament and alveolar bone. In all specimens, the expression of the investigated matrix molecules was stronger at the reformed sites than at the original sites. Osteopontin was present in all specimens in both the regenerated and intact (original) periodontal ligament connective tissue. The staining appeared to be stronger within the regenerated tissues than in the intact ones. The most intense staining was observed near the newly formed cementum and bone. The negative control did not reveal any labeling for osteopontin. In both the regenerated and intact periodontium, collagen I and collagen III were localized throughout the entire periodontal ligament connective tissue. In the regenerated periodontal ligament, collagen III displayed a more intense staining than collagen I. Intracellular staining for collagen III was predominantly observed in the cells lining the new cementum and the new bone. In the intact, non-treated sites of the periodontium both collagen I and collagen III displayed fainter staining than at regenerated sites

(continued)

Table 3.3 (continued)

Reference	Defect type/n subjects	Treatment evaluated	Study length	Analysis	Outcome
Sculean et al. (2003a)	12 advanced intrabony defects (1 and 2-wall) in teeth scheduled for extraction due to advanced periodontal destruction and/or further prosthetic considerations /12 patients suffering from advanced chronic periodontitis	1. GTR (Resolut) 2. EMD 3. EMD + natural bone mineral (NBM) (BioOss)	6 months	H, IHC	The histologic evaluation revealed that the healing resulted to a varying extent in the formation of new cementum, new PDL and new alveolar bone. The newly formed cementum was always in continuation with the old cementum apical to the defect and was of a mixed acellular and cellular type with inserting collagen fibers oriented perpendicularly to the tooth surface. No differences in the quality of the newly formed cementum were found between the treatments. In all monkey and human specimens, the downgrowth of epithelium stopped at the coronal level of newly formed cementum. In all four defects treated with EMD + NBM, the NBM particles were surrounded by a bone-like tissue. No direct contact between the graft material and the tooth surface (dentin or cementum) was observed. The original PDL in both monkeys and humans labeled for vimentin in all specimens studied. The newly formed PDL was always in continuation with the intact PDL present at the apical portion of the defects and labeled in all monkey and human specimens for vimentin. Differences in labeling intensity were neither found between the original and the newly formed PDL nor between monkeys and humans. Labeling for vimentin was also found in the cementoblasts along the newly formed cementum and in the cementocytes included into it

Sculean et al. (2003d)	16 advanced intrabony defect around teeth or roots scheduled for extraction in 16 patients	1. Scaling and root planing with hand instruments and application of EMD (4 defects) 2. Scaling with an ultrasonic instrument and application of EMD (6 defects) 3. Scaling with an ultrasonic instrument alone (6 defects)	6 months	H	Clinical examination revealed a PD reduction and a gain of CAL after all 3 treatment modalities. The histological evaluation, however, revealed that healing in all 3 procedures was predominantly characterized by formation of a long junctional epithelium along the instrumented root surface and no predictable regeneration of attachment apparatus. A minute amount of reparative cementum was observed only occasionally and was always limited to the most apical part of the defect. Within its limits, the study failed to show periodontal regeneration in advanced human intrabony defects following nonsurgical treatment with subgingival application of EMD
Sculean et al. (2005b)	18 advanced intrabony defect around teeth scheduled for extraction in 18 patients	SRP followed by: 1. GTR 2. EMD 3. EMD + BG 4. BDX + GTR 5. BDX 6. EMD + BDX	6 months	H	The healing following all six different types of regenerative treatment resulted to a varying extent in formation of cementum, periodontal ligament and bone. Neither ankylosis nor root resorption was observed. The new cementum displayed a predominantly cellular character and comparable thickness in all six treatment groups. Collagen fibers were observed to run parallel but also to insert into the newly formed cementum, irrespective of the treatment. Artifacts were observed in all biopsies

(continued)

Table 3.3 (continued)

Reference	Defect type/ <i>n</i> subjects	Treatment evaluated	Study length	Analysis	Outcome
Sculean et al. (2008c)	10 advanced combined 1- and 2-wall intrabony defects around teeth scheduled for extraction because of advanced chronic periodontitis and further prosthodontic considerations in 10 nonsmoking subjects (six females and four males), aged between 27 and 62 years (mean age, 48.6 years)	Full-thickness mucoperiosteal flap + 24% EDTA gel 2 min + EMD + a mixture of EMD and biphasic calcium phosphate (BCP)	9 months	H	The clinical measurements demonstrated a reduction in mean PD from 8.6–1.9 mm at baseline to 5.3–2.0 mm at 9 months. The mean CAL changed from 10.8–2.0 mm at baseline to 7.8–1.7 mm at 9 months. Mean CAL gain measured 3.0–1.6 mm. In six of nine biopsies, the histologic findings indicated the formation of cementum with inserting collagen fibers to a varying extent. The newly formed cementum was of amixed acellular and cellular type in all specimens. Collagen fibers were inserting into the newly formed cementum in all specimens showing new attachment. In three of the nine specimens, healing resulted in a long junctional epithelium extending to the bottom of the defect. Mean new connective tissue attachment (i.e., new cementum with inserting collagen fibers) varied from 0.0 to 2.1 mm. The amount of newly formed bone was limited and varied from 0.0 to 0.7 mm. In most specimens, BCP graft particles were encapsulated in connective tissue, whereas formation of a bone-like tissue around the graft particles was observed only occasionally. Direct contact between the graft particles and the root surface (cementum or dentin) was not observed in any of the analyzed specimens
Sculean et al. (2008a)	11 deep intrabony defects in 11 healthy patients all with advanced chronic periodontitis	Full-thickness mucoperiosteal flap + a combination of EMD and a natural bone mineral (NBM)	5 years	H	Mean PD, GR and CAL were significantly reduced at 1 year and at 5 years versus baseline values. Histologic analysis of a mandibular second molar, extracted 5 years after treatment with EMD + NBM, demonstrated bone formation around the NBM particles
St George et al. (2006)	2 intrabony 2-walled defects in 2 patients with chronic periodontitis	Full-thickness mucoperiosteal flap + 24% EDTA 1 min + EMD	6 and 24 months	H	External inflammatory root resorption of the treated teeth

Yukna and Mellonig (2000)	10 intrabony defects in 8 patients with advanced adult periodontitis	Full-thickness flap + citric acid (pH = 1, 1 min) + EMD	6 months	H	The 10 defects studied yielded a total of 3 with regeneration, 3 with new attachment and 3 with long junctional epithelium histologic outcomes. The 3 cases demonstrating regeneration showed connective fiber insertion into both new bone and new cementum. A parallel arrangement of the connective fibers of the PDL could also be observed. Both acellular and cellular new cementum were identified, deposited on both old cementum and dentin. No evidence of root resorption, ankylosis or untoward inflammation was seen. The results of this study fulfill the proof of principle that use of EMD can result in periodontal regeneration on previously diseased root surfaces in humans, but on an inconsistent basis
Windisch et al. (2002)	14 advanced intrabony defects at teeth scheduled for extraction	1. GTR using bioabsorbable barriers 2. EMD	6 months	H	Histometrically, significant amounts of new connective tissue attachment (i.e., cementum with inserting collagen fibers) were observed in both groups (GTR: 2.29 mm; EMD: 1.81 mm). Bone regeneration was found to be significant only in the GTR group (GTR: 1.93 mm; EMD: 0.78 mm). However, the study lacked statistical power for determining equivalence between the groups

PD probing depth, *CAL* clinical attachment level, *EMD* Emdogain, *H* histology, *IHC* immunohistochemistry, *BG* plus bioactive glass, *BDX* bovine-derived xenograft, *GTR* guided tissue regeneration

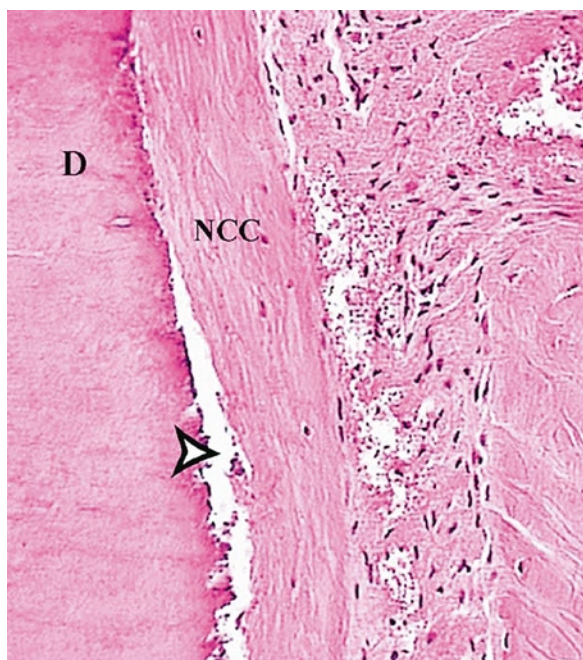


Fig. 3.1 Photomicrograph of newly formed cellular cementum (NCC) following treatment with enamel matrix protein derivative (EMD). Arrowhead indicates the presence of an artifact. *D* dentin. Original magnification $\times 350$ (Sculean et al. 2005b. Reprinted with permission from Springer)

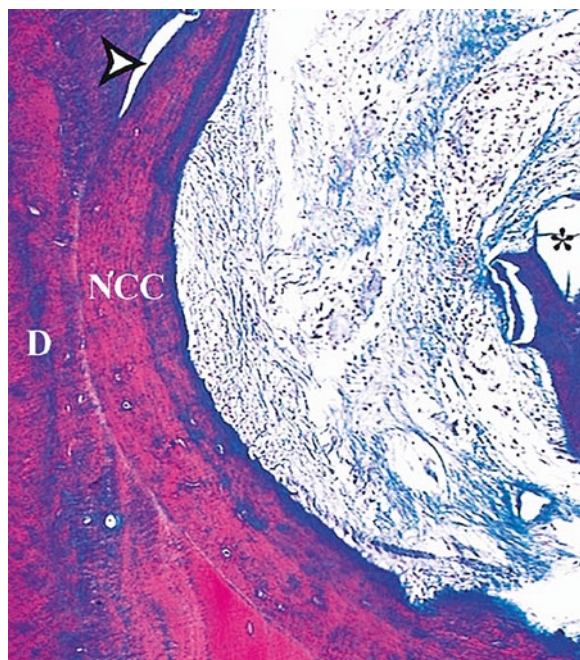


Fig. 3.3 Photomicrograph of newly formed cellular cementum (NCC) following treatment with enamel matrix protein derivative + bovine-derived xenograft (EMD + BDX). Arrowhead indicates the presence of an artifact. *D* dentin, * BDX particle. Original magnification $\times 350$ (Sculean et al. 2005b. Reprinted with permission from Springer)

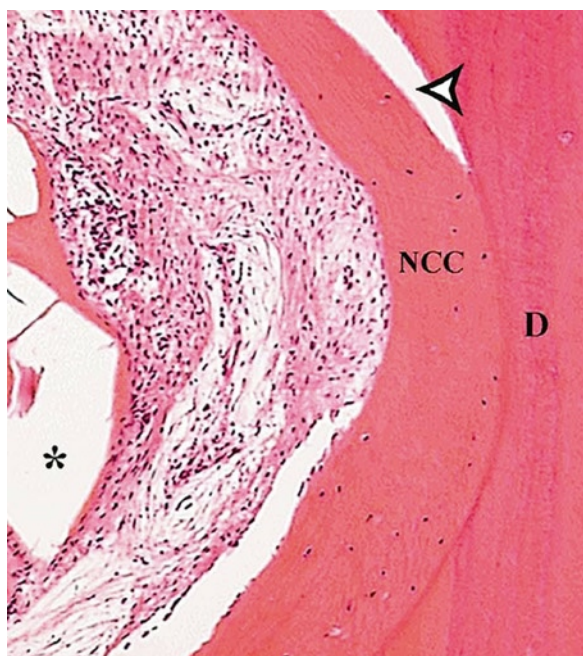


Fig. 3.2 Photomicrograph of newly formed cellular cementum (NCC) following treatment with enamel matrix protein derivative plus bioactive glass (EMD + BG). Arrowhead indicates the presence of an artifact. *D* dentin, * BG particle. Original magnification $\times 350$ (Sculean et al. 2005b. Reprinted with permission from Springer)

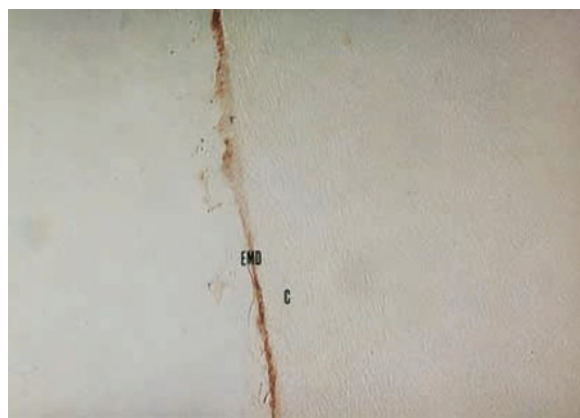


Fig. 3.4 Root surface at 1 week following treatment with an enamel matrix protein derivative (EMD). An intensive immunohistochemical staining can be observed on the cementum (C) surface indicating the presence of EMD. Original magnification $\times 400$ (Sculean et al. 2002c. Reprinted with permission from Springer)

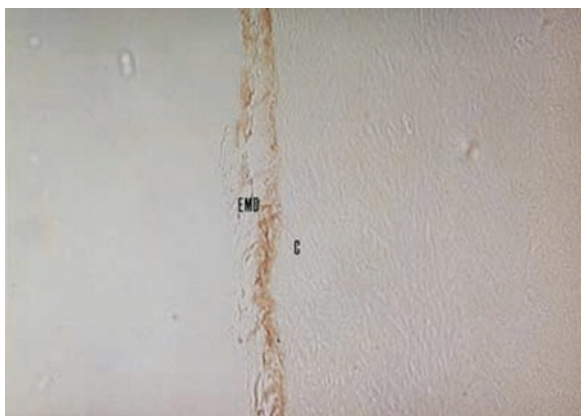


Fig. 3.5 Root surface at 4 weeks following treatment with EMD. The immunohistochemical staining indicates the presence of EMD. C cementum. Original magnification $\times 400$ (Sculean et al. 2002c. Reprinted with permission from Springer)

3.5 Summary of Studies Evaluating the Efficacy of Emdogain in the Treatment of Infrabony Pockets (Vertical Bone Loss)

3.5.1 Nonsurgical Periodontal Therapy

Histological findings have shown that treatment of intrabony defects with nonsurgical periodontal therapy and EMD can result in formation of a long junctional epithelium along the instrumented root surface and no predictable regeneration of attachment apparatus (Sculean et al. 2003d).

Short- and long-term clinical trials have also failed to reveal any additional benefit of EMD as adjunct to nonsurgical periodontal therapy (Gutierrez et al. 2003; Mombelli et al. 2005; Giannopoulou et al. 2006; Wennström and Lindhe 2002).

3.5.2 Surgical Periodontal Therapy

The long-term effects of enamel matrix derivative in the treatment of intrabony defects with surgical periodontal therapy (Fig. 3.6) were evaluated in a number of case reports and controlled clinical trials and summarized by several reviews and meta-analyses (Cochrane reviews: Esposito et al. 2003, 2004, 2005, 2009; Trombelli et al. 2002b; Trombelli and Farina 2008; Giannobile and

Somerman 2003; Kalpidis and Ruben 2002; Venezia et al. 2004). In these systematic reviews and meta-analyses, summarized in Table 3.4, the study population was extended to patients affected by periodontitis with intraosseous defects to be treated. The clinical attachment level change was regarded as the primary outcome measure. Changes in probing depth, gingival recession (GR) and radiographic bone level were also considered. Evaluation of long-term benefits included ease of maintenance based on residual probing depth, incidence of relapsing or recurrent disease and tooth loss. Changes in aesthetic appearance, postoperative complications (infection, soft tissue dehiscences), pain, tooth hypersensitivity, cost/benefit and patient well-being were considered as patient-centered outcomes (Trombelli 2005).

3.5.2.1 Comparison EMD Alone Versus Control/Placebo/Open Flap Debridement

Several recent systematic reviews compared EMD with periodontal flap operation in the treatment of infrabony lesions (Kalpidis and Ruben 2002; Venezia et al. 2004, Esposito et al. 2005, 2009; Tu et al. 2008).

Sculean et al. (2007c) revealed that most data from controlled clinical studies indicate that the additional application of EMD in the context of surgical therapy of deep intrabony periodontal defects may lead to significantly higher gains of clinical attachment and defect fill compared to open flap debridement (OFD) (Froum et al. 2001; Heijl et al. 1997; Okuda et al. 2000; Pontoriero et al. 1999; Sculean et al. 2001b; Silvestri et al. 2000, 2003; Tonetti et al. 2002; Wachtel et al. 2003; Zucchelli et al. 2002). Surgical treatment with EMD was also demonstrated to significantly improve supracrestal soft tissue density compared to open flap debridement alone. In this analysis, performed with computer-assisted densitometry of underexposed radiographs, control sites displayed a significant decrease of tissue density over the first 6 postoperative weeks, with soft tissue density reaching above the baseline values by week 12. EMD-treated sites, however, displayed a significantly different response: already at week 2 the average soft tissue density was above baseline and by week 6 had reached the values observed in both test and controls by week 12. Differences between test and control tissue densities were significant over the initial 6 weeks. These data support the clinical observation that EMD-treated sites display a more rapid healing with little clinically evident inflammation (Tonetti et al. 2004a, b).



Fig. 3.6 Microsurgical access flap for buccally located defects. (a) Site treated with Emdogain® alone and micro Surgically modified “papilla-preservation” technique, with 7 mm loss of attachment before surgery; (b) incision extended beyond the defect border in comparison with the original technique (Cortellini et al. 1995); (c) flap deflection with buccal one + two-wall bony

defect; (d) application of Emdogain; (e) flap reposition and fixation with micro suture (Vicryl 8/0 Ethicon, needle GS-9); and (f) situation 1 year after surgery with improved papilla contour in comparison with preoperative situation (Bokan et al. 2006. Reprinted with permission from John Wiley & Sons)

The data indicate that the clinical outcomes after treatment of intrabony defects with EMD when used either alone or in association with bioactive glass can be maintained up to 10 years (Sculean et al. 2001b, 2002a,

2003b, 2004, 2006, 2007a, 2007d, 2008b; Heden and Wennström 2006; Francetti et al. 2004, 2005).

The aim of the systematic review performed by Tonetti et al. (2002) was to determine the effect of the

Table 3.4 Systematic reviews and meta-analysis on the efficacy of enamel matrix derivative in the treatment of infrabony defects

Authors	Studies included	Conclusions
Trombelli et al. (2002b)	5 RCTs included of at least 6-month duration. Recent search: June 2001	Review of randomized controlled trials of at least 6-month duration was conducted comparing grafting biomaterials/biological agents (alone or in combination) + OFD (test group) to OFD alone or in combination with a placebo (control group). The difference in CAL change between test and control groups varied from -1.45 to 1.40 mm with respect to different biomaterials/biological agents. Meta-analysis showed that CAL (weighted mean difference 1.33 mm, 95% CI: 0.78-1.88) and PD (weighted mean difference 1.60 mm, 95% CI: 0.59-2.62) significantly improved after treatment for enamel matrix proteins
Kalpidis and Ruben (2002)	12 studies that included 317 infrabony defects were analyzed. The observation period was more than 6 month duration	CAL gain amounted to 3.2 ± 0.9 mm (33% of the original CAL) and PD reduction averaged 4.0 ± 0.9 mm (50% of the baseline PD) for a total of 317 lesions with a mean baseline depth of 5.4 ± 0.8 mm. Improvements in clinical parameters achieved with EMD were statistically significant in reference to preoperative measurements. Similar therapeutic results were reported in studies where EMD was compared directly to GTR. The statistical superiority of EGR over treatment with open flap debridement has been established
Giannobile and Somerman (2003)	8 studies, representing 7 RCTs and 1 quasi-experimental study, representing a total population of 511 subjects were analyzed. Most recent search: April 2002 The observation period was more than 8-month duration	(1) There is evidence supporting the use of EMD for periodontal osseous defects to improve CAL and reduce PD, although long-term benefits have not been established. (2) EMD has demonstrated notable consistency among the studies investigated in terms of superiority to controls (in general compared to open flap debridement OFD). (3) EMD appears to be safe for single and multiple administrations in terms of lack of elicitation of antibody responses or other local/systemic inflammatory events

(continued)

Table 3.4 (continued)

Authors	Studies included	Conclusions
Esposito et al. (2003)	10 trials with at least 1 year of follow-up that enrolled 577 patients. Most recent search: January 2003	No difference in tooth loss was observed. A meta-analysis including eight trials showed that Emdogain treated sites displayed statistically significant CAL improvements (mean difference 1.3 mm, 95% CI: 0.8–1.8) and PD reduction (1 mm, 95% CI: 0.5–1.4) when compared to flap surgery. Comparing EMD with GTR (six trials), GTR showed a statistically significant reduction of PD (0.6 mm) and increase of REC (0.5 mm). No difference in postoperative infections was observed. It was concluded that EMD is able to significantly improve PAL levels (1.3 mm) and PPD reduction (1 mm) when compared to flap surgery; however these results may not have a great clinical impact, since it has not been shown that more periodontally compromised teeth could be saved. There was no evidence of clinically important differences between GTR and Emdogain
Venezia et al. (2004)	28 studies that included 955 intrabony defects were analyzed. The clinical evaluation was performed following a period of at least 6 months, except for one study included. Most recent search: May 2003	The meta-analysis of the effect of EMD in intrabony defects with 28 studies that included 955 intrabony defects, revealed that the mean initial probing depth of 7.94 ± 0.05 mm was reduced to 3.63 ± 0.04 mm ($P=0.000$) following treatment with EMD. The mean CAL changed from 9.4 ± 0.06 to 5.82 ± 0.07 mm ($P=0.000$). The mean gingival recession increased from 1.31 ± 0.03 to 2.4 ± 0.06 mm ($P=0.000$). The meta-analysis for the reentry studies resulted in a mean defect fill of 3.78 ± 0.03 mm and a mean crestal bone resorption of 0.46 ± 0.01 mm. The meta-analysis for the radiographic data resulted in a mean defect resolution of 2.02 ± 0.08 mm and a bone gain of 2.37 ± 0.17 mm. The authors concluded that EMD seems to be safe, was able to regenerate lost periodontal tissues in previously diseased sites based on clinical parameters and was better than OFD or GTR. Its combination with allograft materials may be of additional benefit but still needs to be further investigated

Esposito et al. (2005)	10 RCTs were included out of 29 potentially eligible trials. All data refer to the 1-year time point. Most recent search: May 2005	A meta-analysis including eight trials showed that EMD treated sites displayed statistically significant PAL improvements (mean difference 1.2 mm, 95% CI 0.7–1.7) and PPD reduction (0.8 mm, 95% CI 0.5–1.0) when compared to placebo or control-treated sites, though a high degree of heterogeneity was found. Significantly more sites had <2 mm PAL gain in the control group, with RR 0.48 (95% CI 0.29–0.80). Approximately six patients needed to be treated (NNT) to have one patient gaining 2 mm or more PAL over the control group, based on a prevalence in the control group of 35%. No differences in tooth loss or aesthetic appearance as judged by the patients were observed. When evaluating the only two trials at a low risk of bias in a sensitivity analysis, the effect size for PAL was 0.6 mm, which was less than 1.2 mm for the overall result. Comparing EMD with GTR (five trials), GTR showed a statistically significant increase of REC (0.4 mm) and significantly more postoperative complications. No trials were found comparing EMD with BG
Trombelli and Farina (2008)	Different study designs and methodology that provide different levels of evidence were considered: proof-of-principle (descriptive) studies, randomized clinical trials (RCTs) and/or systematic reviews (SRs). Most recent search: December 2007	The purpose of the present review was to determine the clinical effect of the use of bioactive agents for the treatment of intraosseous and furcation defects. The review indicated that: (1) EMD either alone or in combination with grafts can be effectively used to treat intraosseous defects and the clinical results appear to be stable long term and (2) the additional use of a graft seems to enhance the clinical outcome of EMD
Tu et al. (2008)	10 RCTs over 12 months duration. Most recent search: January 2003	Meta-analysis conducted to investigate whether or not there was a temporal trend in the treatment efficacy reported in the RCTs on EMD in the treatment of infrabony defects. Despite the small sample size, the treatment effect of EMD and flap operation in CAL gain showed a positive (0.128 and 0.115 mm per year, respectively) temporal relationship. It was suggested that only RCTs should be included in the meta-analysis of GTR and EMD, as the treatment effect of the control group cannot be assumed to be constant

(continued)

Table 3.4 (continued)

Authors	Studies included	Conclusions
Esposito et al. (2009)	13 RCTs were included out of 35 potentially eligible trials. All data refer to the 1-year time point. Most recent search: February 2009	The meta-analysis revealed that 1 year after its application, EMD significantly improved CAL levels (1.1 mm) and PD reduction (0.9 mm) when compared to a placebo or control; however, the high degree of heterogeneity observed among trials suggests that results have to be interpreted with great caution. In addition, a sensitivity analysis indicated that the overall treatment effect might be overestimated. The actual clinical advantages of using EMD are unknown. With the exception of significantly more postoperative complications in the GTR group, there was no evidence of clinically important differences between GTR and EMD. Bone substitutes may be associated with less REC than EMD
Tu et al. (2010)	28 RCTs at least 6 months duration. Most recent search: December 2008	Network meta-analyses of randomized-controlled trials were undertaken to investigate whether EMD in conjunction with other regenerative materials yield better treatment outcomes than EMD alone in the treatment of infrabony defects ≥ 3 mm. EMD plus bone grafts and EMD plus membranes attained 0.24 mm (95% HPD intervals: -0.38, 0.65) and 0.07 mm (95% HPD intervals: -1.26, 1.04) more PD reduction than EMD alone, respectively. For CAL gain, EMD plus bone grafts and EMD plus membranes attained 0.46 mm (95% HPD intervals: -0.17, 0.83) and 0.15 mm (95% HPD intervals: -1.37, 0.30), respectively. When different types of bone grafts and barrier membranes were treated separately, EMD with bovine bone grafts showed greater treatment effects. It was concluded that there was little evidence to support the additional benefits of EMD in conjunction with other regenerative materials

CAL clinical attachment level, PD probing depth, REC recession, GTR guided tissue regeneration, EMD enamel matrix derivative, HPD high probability density

use of grafting biomaterials or the application of biological agents in addition to conventional open flap debridement (OFD) in the treatment of deep intraosseous defects. Specifically, the additional efficacy of biomaterials or biological agents in comparison to OFD only procedure was evaluated on short-term and long-term clinical and patient-centered outcome parameters. The estimate of the effect of the treatment with EMD, as assessed in five studies (Froum et al. 2001; Okuda et al. 2000; Pontoriero et al. 1999; Silvestri et al. 2000; Tonetti et al. 2002), showed a greater CAL gain of 1.33 mm (95% CI: 1.01–1.42; Q-test for heterogeneity: 24.27 (df=4), $P < 0.001$) when compared with control procedure ($P < 0.001$). However, analysis contained statistically significant heterogeneity in results among studies. The Galbraith plot shows that the results from Silvestri et al. (2000) were highly inconsistent with the overall estimate. Meta-analysis regression was undertaken to explore if heterogeneity could be accounted for initial defect depth in both test and control groups. Results showed no evidence of an effect of the predictor on difference in CAL gain between groups ($P = 0.81$). Initial defect depth could therefore not account for this variability. Meta-analysis showed also that EMD resulted in significantly greater PD reduction when compared with OFD procedure. Weighted mean difference was 1.60 mm (95% CI: 0.59–2.62; P -value for estimate = 0.002; P -value for heterogeneity < 0.001).

The meta-analytic results from 12 controlled studies (Heijl et al. 1997; Pontoriero et al. 1999; Sculean et al. 1999a; Lekovic et al. 2000; Okuda et al. 2000; Silvestri et al. 2000; Froum et al. 2001; Pietruska 2001; Sculean et al. 2001c; Zucchelli et al. 2002; Velasquez-Plata et al. 2002; Tonetti et al. 2002) on a total of 317 compiled intrabony defects treated with EMD were analyzed by Kalpidis and Ruben (2002). The angular bone defects had an overall intraosseous vertical destruction of 5.4 ± 0.8 mm measured during surgery. EMD treatment resulted in a residual pocket 3.9 ± 0.8 mm deep, compared with to the initial depth of 7.9 ± 0.8 mm. This probing difference corresponds to a compiled pocket reduction of 4.0 ± 0.9 mm and a relative percent PD decrease of $50.4 \pm 9.9\%$ for a total of 317 lesions in 284 patients. Topical application of EMD improved CAL from 9.4 ± 1.1 mm at baseline to 6.3 ± 1.0 mm, indicating 3.2 ± 0.9 mm of attachment gain and a relative $32.7 \pm 7.6\%$ CAL improvement. Considering soft tissue marginal levels, a minimal additional recession of 0.9 ± 0.4 mm was attributed to

the surgical application of EMD. Management of intrabony deformities with OFD alone produced a mean attachment gain of 2.1 mm (22.4%) and a pocket reduction of 3.1 mm (40.1%) (Kalpidis and Ruben 2002).

The systematic review performed by Giannobile and Somerman (2003) evaluated the evidence to support the utilization of EMD and growth factors for periodontal repair and regeneration associated with natural teeth. Eight studies, representing seven randomized controlled trials (RCTs) (Heijl et al. 1997; Pontoriero et al. 1999; Okuda et al. 2000; Silvestri et al. 2000; Froum et al. 2001; Sculean et al. 2001c; Tonetti et al. 2002) and one quasi-experimental study (Zetterström et al. 1997), representing a total population of 511 subjects were analyzed with respect to EMD. The authors concluded that there was evidence supporting the use of EMD for periodontal osseous defects to improve CAL and reduce PD, although long-term benefits have not been established, that EMD has demonstrated notable consistency among the studies investigated in terms of superiority to controls (in general compared to open flap debridement) and that EMD appears to be safe for single and multiple administrations in terms of lack of elicitation of antibody responses or other local/systemic inflammatory events.

Venezia et al. (2004) performed a meta-analysis of the effect of EMD in intrabony defects with 28 studies that included 955 intrabony defects. The mean initial probing depth of 7.94 ± 0.05 mm was reduced to 3.63 ± 0.04 mm ($P = 0.0001$) following treatment with EMD. The mean clinical attachment level changed from 9.4 ± 0.06 to 5.82 ± 0.07 mm ($P = 0.0001$). The mean gingival recession increased from 1.31 ± 0.03 to 2.4 ± 0.06 mm ($P = 0.0001$). The meta-analysis for the reentry studies resulted in a mean defect fill of 3.78 ± 0.03 mm and a mean crestal bone resorption of 0.46 ± 0.01 mm. The meta-analysis for the radiographic data resulted in a mean defect resolution of 2.02 ± 0.08 mm and a bone gain of 2.37 ± 0.17 mm (Venezia et al. 2004). The meta-analysis results obtained following the treatment with EMD were compared with those obtained following open flap debridement procedures (eight studies: Heijl et al. 1997; Zetterström et al. 1997; Pontoriero et al. 1999; Okuda et al. 2000; Silvestri et al. 2000; Sculean et al. 2001c; Tonetti et al. 2002; Zucchelli et al. 2002). No significant difference was found in the mean initial probing depth between the EMD group and the OFD group ($P = 0.849$). However, the

probing depth reduction following the treatment with EMD was significantly higher in the EMD group (4.82 ± 0.02 mm vs. 2.59 ± 0.06 mm, $P=0.0001$). Similar results were obtained for the clinical attachment level (CAL) results. Although no significant difference was found in the mean initial CAL between the EMD group and the OFD group ($P=0.579$), better clinical attachment gain was obtained in the EMD group (4.07 ± 0.03 mm vs. 2.55 ± 0.04 mm, $P=0.0001$). Considering the calculated change in the free gingival margin location, no differences were noted between the two groups at the initial examination ($P=0.555$) and lower recession was found following the treatment with EMD (0.77 ± 0.02 mm vs. 1.37 ± 0.04 mm, $P=0.0001$) (Venezia et al. 2004).

Esposito et al. (2009) evaluated nine trials (Francetti et al. 2004; Grusovin et al. 2009; Heijl et al. 1997; Okuda et al. 2000; Pontoriero et al. 1999; Rösing et al. 2005; Silvestri et al. 2000; Tonetti et al. 2002; Zucchelli et al. 2002) that compared EMD versus control flap surgery. The surgical techniques for the control flaps were the modified Widman flap in four trials (Heijl et al. 1997; Okuda et al. 2000; Pontoriero et al. 1999; Silvestri et al. 2000), whereas in the other five trials (Francetti et al. 2004; Grusovin et al. 2009; Rösing et al. 2005; Tonetti et al. 2002; Zucchelli et al. 2002) the simplified or the modified papilla preservation techniques were used. In five trials (Grusovin and Esposito 2009; Heijl et al. 1997; Okuda et al. 2000; Pontoriero et al. 1999; Rösing et al. 2005), a placebo (the propylene glycol alginate vehicle gel solution) was used in the control flaps. The meta-analysis showed a significant gain in mean CAL for EMD compared with control sites with mean difference of 1.08 mm (95% CI: 0.61–1.55, $\chi^2=38.10$, 8 df, $P<0.00001$, $I^2=79\%$). It was also revealed a significant reduction in mean PD for EMD compared with control sites with mean difference of 0.88 mm (95% CI: 0.44–1.31; $\chi^2=25.43$, 8 df, $P=0.001$, $I^2=69\%$). However, there was no statistically significant difference between the EMD and the control in REC ($P=0.13$; $I^2=41\%$) as well as for radiographic bone gain ($P=0.01$; $I^2=78\%$).

Although EMD initially gave rise to a great hope and expectation in achieving regeneration of lost periodontal tissue due to encouraging results from several case reports, recently published systematic reviews of randomized controlled trials (RCTs) (Esposito et al.

2005, 2009), however, showed that in general EMD achieved only modestly better results compared with traditional flap operations. Furthermore, they raised the issue of whether these expensive treatments are actually cost effective (Esposito et al. 2009). Tu et al. (2008) conducted a meta-analysis to investigate whether or not there was a temporal trend in the treatment efficacy reported in the RCTs on EMD in the treatment of infrabony defects. Ten studies (Heijl et al. 1997; Pontoriero et al. 1999; Okuda et al. 2000; Silvestri et al. 2000; Tonetti et al. 2002; Zucchelli et al. 2002; Francetti et al. 2004, 2005; Rösing et al. 2005; Bokan et al. 2006) reporting PD and CAL for EMD were analyzed using weighted ordinary regression. In terms of PD reduction, a small nonsignificant negative temporal relationship was observed between treatment difference and publication years (-0.075 mm per year [-0.266 , 0.116]; $P=0.390$). However, positive but nonsignificant temporal relationships were observed in treatment effectiveness for the EMD and control groups (0.083 mm per year [-0.082 , 0.249] and 0.166 mm per year [-0.026 , 0.358]; $P=0.279$ and 0.082 , respectively). In terms of CAL gain, a very small nonsignificant positive relationship was observed between treatment difference and publication years (0.014 mm per year [-0.182 , 0.210]; $P=0.871$). Positive but nonsignificant temporal relationships were observed in treatment effectiveness for EMD and control groups (0.128 mm per year [-0.078 , 0.334] and 0.115 mm per year [-0.016 , 0.246]; $P=0.189$ and 0.078 , respectively). However, despite the small sample size, the treatment effect of EMD and flap operation in CAL gain showed a positive (0.128 and 0.115 mm per year, respectively) temporal relationship. A previous meta-analysis on the fading of reported effectiveness found that baseline values were the most important predictors of effect size (Gehr et al. 2006) and up to 80% of effect size variability can be explained by baseline CAL and PD values. It was suggested that only RCTs should be included in the meta-analysis of GTR and EMD, as the treatment effect of the control group cannot be assumed to be constant. Therefore, systematic reviews/meta-analyses with a surgical control group may need to explore whether the treatment effect of control group improves over time, and this may help to evaluate the efficacy of a surgical test procedure (Tu et al. 2008).

3.5.2.2 Comparison EMD Alone Versus GTR

Histologically, a clear advantage for GTR is evident compared with EMD. Almost all of the GTR-treated defects are characterized by true periodontal regeneration to some degree (Sculean et al. 1999b; Windisch et al. 2002). In contrast, EMD-treated defects are generally characterized by new attachment that is not always followed by bone regeneration (Venezia et al. 2004).

Meta-analysis, reported by Kalpidis and Ruben (2002), of data from control therapies in EMD studies revealed an attachment improvement of 3.8 mm (38.7%) and a pocket reduction of 5.1 mm (60.9%) in 112 intrabony defects treated with occlusive membranes. Despite considerable variation observed for all therapies, both EMD and GTR resulted in statistically significant superior outcomes compared to OFD. Meta-analysis of data revealed a difference of 0.6 mm (18%) in CAL gain between EMD and GTR, in favor of GTR treatment. The 18% difference suggests that the GTR clinical outcomes may be superior compared to those reported after EMD treatment. The authors recommended to interpret this conclusion cautiously because it was not based on matched patient populations. Moreover, this difference seems to be clinically insignificant considering that GTR is technique sensitive, highly susceptible to bacterial insult and associated with higher morbidity when non-resorbable membranes are utilized (Kalpidis and Ruben 2002).

A meta-analysis comparison of the results for EMD and GTR performed by Venezia et al. (2004) found no statistically significant difference between the mean initial probing depth of the 2 groups, the mean probing depth reduction was higher in the GTR group (4.82 ± 0.02 mm vs. 5.24 ± 0.13 mm). In contrast, while no statistically significant difference was found between the mean initial CAL, CAL gain was higher for the EMD (4.07 ± 0.03 mm vs. 3.64 ± 0.12 mm). As expected, these discrepancies were resolved because of the greater increase in recession in the GTR group (0.77 ± 0.02 mm vs. 1.5 ± 0.16 mm) (Venezia et al. 2004).

The six trials (Crea et al. 2008; Pontoriero et al. 1999; Sanz et al. 2004; Silvestri et al. 2000, 2003; Zucchelli et al. 2002) evaluated in the review performed by Esposito et al. (2009) compared EMD

versus guided tissue regeneration (GTR). In four trials non-resorbable barriers were used (Crea et al. 2008; Silvestri et al. 2000; Silvestri et al. 2003; Zucchelli et al. 2002), in one trial resorbable barriers were used (Sanz et al. 2004), and in one trial (Pontoriero et al. 1999) both resorbable and non-resorbable barriers were used. The meta-analysis revealed that there were no statistically significant differences between the two procedures regarding CAL gain, PD reduction and changes in radiographic bone level. However, there were significant differences between EMD and GTR for change from baseline in REC (five trials), with a significant increase in recession for GTR with mean difference 0.41 mm (95% CI: 0.15–0.66; $\chi^2=3.10$, 4 df, $P=0.54$). There were statistically significant more postoperative complications in the GTR group (three trials; $P=0.03$), RR=0.12 (95% CI: 0.02–0.85).

3.5.2.3 Comparison EMD Versus Bone Graft

The comparison of the results obtained following treatment with EMD with those obtained following bovine-derived apatitic xenograft (BDX) revealed a higher initial probing depth (7.94 ± 0.05 vs. 7.38 ± 0.23 , $P<0.001$) and probing depth reduction (4.82 ± 0.02 vs. 4.5 ± 0.28 , $P<0.001$) in the EMD group, along with higher CAL gain (although not significant) and higher increase in gingival recession (0.77 ± 0.02 vs. 0.5 ± 0.13 , $P=0.001$). The author of this meta-analysis Venezia et al. (2004) recommended to consider this comparison with extra caution, since only 2 studies were eligible for the meta-analysis.

In the review performed by Esposito et al. (2009), only one trial (Leknes et al. 2009) that compared EMD versus a bioactive glass (PerioGlas, US Biomaterials, Alachua, FL, USA) was retained for analysis. Thirteen chronic periodontitis patients, 41–74 years of age, who had two proximal intrabony defects in different jaw quadrants with ≥ 3 mm vertical radiographic bone loss, were selected. After initial therapy, the sites in each patient were randomly assigned to EMD or bioactive glass treatment. Clinical attachment level (CAL), probing depth (PD), tooth mobility, gingival recession (GR), bleeding on probing and dental plaque were recorded at baseline and at 6 and 12 months. Bioactive glass treatment resulted in a significant gain in proximal CAL (1.0 ± 1.1 mm, $P=0.005$) and a

reduction in proximal PD at 6 months (2.4 ± 1.5 mm, $P < 0.001$), but there was no further improvement from 6 to 12 months (CAL change_{6-12 months}: 0.2 ± 0.8 mm, PD change_{6-12 months}: 0.2 ± 1.3 , $P > 0.05$). Paired comparisons by time for the EMD group revealed a significant reduction in proximal PD at 12 months (2.5 ± 1.9 mm, $P = 0.001$), whereas the gain in proximal CAL approached significance (0.6 ± 1.0 mm, $P = 0.056$). Mean GR increased significantly from baseline to 6 months in both groups (EMD: -1.5 ± 1.2 , Bioactive glass: -1.0 ± 1.0 , $P = 0.001$). The multiple stepwise regression analysis revealed that, within the EMD group, smoking ($P = 0.004$) and TM ($P = 0.016$) negatively influenced the gain of attachment between baseline and 12 months, whereas the PD reduction was negatively influenced by increasing CEJ–BC distance ($P = 0.001$). Within the bioactive glass group, buccal GR increased significantly with age ($P = 0.019$) and increasing CEJ–BC distance ($P = 0.04$), whereas proximal GR increased significantly with age ($P = 0.029$) and increasing MD–W ($P = 0.002$) (Leknes et al. 2009). The authors concluded that the gain in proximal attachment after treating intrabony defects by flap surgery with bioactive glass was significant ($P = 0.004$) and twice that following treatment with EMD (1.2 ± 1.2 mm vs. 0.6 ± 1.0 mm). No differences were noted regarding the proximal PD reduction at 12 months (2.6 ± 1.1 mm vs. 2.5 ± 1.9 mm). Patient and site variables affected the clinical outcome differently (Leknes et al. 2009).

3.5.2.4 Combined Therapies

Although regenerative treatment with EMD offers interesting perspectives to the clinicians, some practical problems were observed. One such problem is the collapse of the mucoperiosteal flap, which occurs frequently in deep one or two-walled defects. This in turn may limit the available space for periodontal regeneration. Thus, both from a biologic as well as a clinical point of view, it was important to evaluate the combination of EMD and a bone graft as a treatment of advanced intrabony defects (Sculean et al. 2002b).

Combination EMD + GTR Versus EMD or GTR alone

The treatment of intraosseous defects with the combination of EMD with GTR has been explored in several clinical studies (Sculean et al. 2001c; 2004; Minabe et al. 2002; Sipos et al. 2005). RCTs evaluating the effectiveness of the combination of EMD with GTR are summarized in Table 3.5.

One broad qualitative systematic review and one network meta-analysis have considered whether or not EMD in conjunction with the uses of barrier membranes and/or bone grafts provide better treatment outcomes than EMD alone (Trombelli and Farina 2008; Tu et al. 2010).

As Trombelli and Farina (2008) reviewed, the clinical trials concerning EMD +GTR application in human intra-osseous defects include both resorbable (Sculean et al. 2001c, 2004; Minabe et al. 2002) and non-resorbable membranes (Sipos et al. 2005). EMD combined with resorbable membranes resulted in a CAL gain ranging from about 3.00 mm (Minabe et al. 2002) to 3.40 mm (Sculean et al. 2001c) at 12 months after surgery. The association of EMD and GTR (resorbable membrane) did not seem to improve the reconstructive outcomes obtained by GTR alone at 1 year postsurgery (Sculean et al. 2001c; Minabe et al. 2002). When compared with EMD alone, the EMD+GTR (resorbable membranes) combination did not show any additional benefit on clinical and radiographic parameters both in the short term (Sculean et al. 2001c, 2004; Minabe et al. 2002) and during maintenance (Sculean et al. 2004).

The network meta-analyses reported by Tu et al. (2010) revealed that EMD plus membranes attained 0.07 mm (95% HPD intervals: -1.26 , 1.04) more PD reduction than EMD alone, respectively. For CAL gain, EMD plus membranes attained 0.15 mm (95% HPD intervals: -1.37 , 0.30), respectively.

Combination of EMD + Different Types of Bone Graft/Bone Substitute Versus EMD or Graft Alone

Several studies have evaluated the effect of a combination of EMD and various types of bone graft/bone

Table 3.5 Summary of RCTs evaluating the effectiveness of enamel matrix derivative (EMD) alone or in association with guided tissue regeneration (GTR) or/and bone grafts with respect to either GTR alone, graft alone or EMD alone

Author, year	Study population	Defect morphology	Treatment modalities	Study length	Outcomes
<i>EMD + GTR versus GTR</i>					
Sculean et al. (2001c)	56 patients, 32 females, age 29–68 year	1, 2, 3-wall intrabony defects of a depth of at least 6 mm	1. EMD placement 2. GTR technique: (Resolut, Gore-Tex, USA); 3. Combination: EMD + GTR; 4. Control: (access flap surgery)	12 months	The sites treated with GTR showed a mean PPD reduction of 4.2 ± 1.9 mm and a mean CAL gain of 3.1 ± 1.5 mm ($P < 0.001$). The sites treated with the combined treatment showed a mean PD reduction of 4.3 ± 1.4 mm and a mean CAL gain of 3.4 ± 1.1 mm ($P < 0.001$). No statistical significant differences in PD reduction and CAL gain were observed between GTR vs. EMD + GTR treatments.
Minabe et al. (2002)	61 patients, 33 females, age 38–62 year	1, 2, 3 walls	1. GTR (tissue guide) 2. EMD 3. EMD + GTR	12 months	The results showed no significant differences in reduction of PD, CAL or radiographic bone gain between the three treatment groups (For GTR: PD change = 3.7 ± 1.2 mm, CAL change = 2.8 ± 0.9 mm; For EMD + GTR: PD change = 4.3 ± 1.6 mm, CAL change = 3.0 ± 1.3 mm). The combination of GTR using a resorbable membrane for intrabony defects and EMD did not enhance the therapeutic effect compared with each monotherapy.
Sculean et al. (2004)	56 patients, 32 females, age 29–68 year (same study population as Sculean et al. 2001)	1, 2, 3-wall intrabony defects of a depth of at least 6 mm	1. EMD placement 2. GTR technique: (Resolut, Gore-Tex, USA); 3. Combination: EMD + GTR; 4. Control: (access flap surgery)	5 years	At 5 years, the PD and CAL were still statistically significantly improved compared with baseline ($P < 0.001$). However, between the groups no statistically significant differences were found (For GTR: PD change = 3.9 ± 1.6 mm, CAL change = 2.7 ± 0.9 mm; For EMD + GTR: PD change = 4.0 ± 1.0 mm, CAL change = 2.6 ± 0.7 mm).

(continued)

Table 3.5 (continued)

Author, year	Study population	Defect morphology	Treatment modalities	Study length	Outcomes
<i>EMD + GTR versus EMD</i>					
Sculean et al. (2001c)	56 patients, 32 females, age 29–68 year	1, 2, 3-wall intrabony defects of a depth of at least 6 mm	1. EMD 2. GTR (Resolut, Gore-Tex, USA); 3. EMD + GTR; 4. Control: access flap surgery	12 months	At 1 year after therapy, the sites treated with EMD demonstrated a mean PPD reduction of 4.1 ± 1.7 mm and a mean CAL gain of 3.4 ± 1.5 mm ($P < 0.001$). The sites treated with the combined treatment showed a mean PD reduction of 4.3 ± 1.4 mm and a mean CAL gain of 3.4 ± 1.1 mm ($P < 0.001$). No statistically significant differences in PD reduction and CAL gain were observed between EMD vs. EMD + GTR treatments.
Sculean et al. (2004)	56 patients, 32 females, age 29–68 year (same study population as Sculean et al. 2001c)	1, 2, 3-wall intrabony defects of a depth of at least 6 mm	1. EMD 2. GTR (Resolut, Gore-Tex, USA); 3. EMD + GTR; 4. Control: access flap surgery	5 years	At 5 years, the PD and CAL was still statistically significantly improved compared with baseline ($P < 0.001$). However, between the groups no statistically significant differences were found (For EMD: PD change = 4.3 ± 1.7 mm, CAL change = 2.9 ± 1.6 mm; For EMD + GTR: PD change = 4.0 ± 1.0 mm, CAL change = 2.6 ± 0.7 mm).
Minabe et al. (2002)	61 patients, 33 females, age 38–62 year	1, 2, 3 walls	1. GTR (Tissue Guide) 2. EMD 3. EMD + GTR	12 months	The results showed no significant differences in reduction of PD, CAL or radiographic bone gain between the three treatment groups (For EMD: PD change = 3.8 ± 0.9 mm, CAL change = 2.6 ± 1.0 mm; For EMD + GTR: PD change = 4.3 ± 1.6 mm, CAL change = 3.0 ± 1.3 mm). The combination of GTR using a resorbable membrane for intrabony defects and EMD did not enhance the therapeutic effect compared with each monotherapy.

Table 3.5 (continued)

Author, year	Study population	Defect morphology	Treatment modalities	Study length	Outcomes
Sipos et al. (2005)	11 patients, 18–55 years	3 walls	1. EMD 2. EMD + GTR (tetracycline-coated e-PTFE)	12 months	After 12 months, the EMD defects showed a significant mean PD reduction of 2.86 ± 0.75 mm, a mean gain in CAL of 1.28 ± 2.04 mm, a mean probing bone level (PBL) gain of 1.63 ± 1.21 mm and a mean increase of recession (REC) of 1.56 ± 2.30 mm. The EMD + GTR defects showed a significant mean PD reduction of 3.02 ± 1.55 mm, a mean gain in CAL of 1.65 ± 1.29 mm, a mean PBL gain of 1.58 ± 1.92 mm and a mean increase of REC of 1.38 ± 1.63 mm. Except for significantly more postoperative discomfort at the EMD + GTR sites, no significant differences were found between EMP and EMD + GTR defects.

substitute in the treatment of intrabony defects (summarized in Table 3.6).

Sculean et al. (2007c) revealed that clinical studies comparing treatment with a combination of EMD and a bone graft/bone substitute to bone graft/bone substitute alone did not demonstrate any advantage of the combination approach (Hoidal et al. 2008; Scheyer et al. 2002; Sculean et al. 2002a, b).

Controlled clinical studies comparing treatment of intrabony defects with EMD alone or a combination of EMD and different types of bone graft/bone substitute (Bokan et al. 2006; Guida et al. 2007; Gurinsky et al. 2004; Jepsen et al. 2008; Kuru et al. 2006; Lekovic et al. 2000; Sculean et al. 2005a, 2005c, 2007a; Velasquez-Plata et al. 2002; Yilmaz et al. 2010; Zucchelli et al. 2003) (Fig. 3.7) seem to indicate that the combination of EMD and demineralized freeze-dried allogenic bone or a natural bone mineral may enhance the clinical outcome (Sculean et al. 2007b, 2007c).

The meta-analysis performed by Venezia et al. (2004) for the results obtained following treatment with EMD with those obtained following combined treatment of EMD and BDX revealed that a higher initial probing depth and probing depth reduction were

found with the EMD group compared with the EMD + BDX group (7.94 ± 0.05 mm vs. 7.32 ± 0.12 mm and 4.82 ± 0.02 mm vs. 3.94 ± 0.11 mm, respectively). The CAL gain was higher for the EMD group (4.07 ± 0.03 mm vs. 3.48 ± 0.12 mm), although the initial CAL measurements were not available for comparison. In addition, the increase in recession was higher in the EMD group (0.77 ± 0.02 mm vs. 0.58 ± 0.06 mm). Similar results were found when EMD was compared with BDX alone: higher initial probing depth and probing depth reduction (4.82 ± 0.02 mm vs. 4.5 ± 0.28 mm) in the EMD group, along with higher CAL gain (4.07 ± 0.03 mm vs. 4.02 ± 0.31 mm) and higher increase in gingival recession (Venezia et al. 2004).

The network meta-analyses reported by Tu et al. (2010) showed that EMD plus bone grafts attained 0.24 mm [95% high probability density (HPD) intervals: $-0.38, 0.65$] more PD reduction than EMD alone, respectively (Fig. 3.8). For CAL gain, EMD plus bone grafts attained 0.46 mm (95% HPD intervals: $-0.17, 0.83$). When different types of bone grafts and barrier membranes were treated separately, EMD with bovine bone grafts showed greater treatment

Table 3.6 Summary of RCTs evaluating the effectiveness of enamel matrix derivative (EMD) alone or in association with bone grafts with respect to either graft alone or EMD alone

Author, year	Study population	Defect morphology	Treatment modalities	Study length	Outcomes
<i>EMD + graft versus graft</i>					
Aspiello et al. (2011)	56 patients, 34 females, age 48–62 years	2, 3 walls	1. EMD + DFDBA 2. DFDBA + saline	12 months	At 12 months, statistically significant differences were found in the test group compared to the control group in PD reduction (5.0 mm vs. 4.0 mm; $P < 0.05$), CAL gain (4.0 mm vs. 3.25 mm) and HTF (4.0 mm vs. 3.5 mm; $P < 0.05$). In the test group, 25% of sites gained >4 mm of CAL, while in the control group, 7.1% of sites gained >4 mm of CAL vs. 3.5 mm; $P < 0.05$). In the test group, 25% of sites gained >4 mm of CAL, while in the control group, 7.1% of sites gained >4 mm of CAL. Both treatments showed a good soft and hard periodontal tissue response.
Hoidal et al. (2008)	32 patients, 13 females, age 31–68 year	1, 2, 3 walls and combination	1. EMD + DFDBA 2. DFDBA	6 months	Both treatment groups resulted in statistically significant PD reduction and CAL gain compared to baseline ($P < 0.001$). However, no statistically significant difference in PD reduction (2.45 ± 0.35 mm vs. 2.56 ± 1.42 mm) or CAL gain (1.63 ± 1.36 mm vs. 1.47 ± 1.40 mm) was found between the two treatment groups. Both treatments also resulted in a statistically significant change in recession compared to baseline ($P < 0.01$); however, no statistically significant difference in recession was found between the treatment groups (2.45 ± 0.35 mm vs. 2.56 ± 1.42 mm). Evaluation of the hard tissue values for defect fill, % defect fill and % defect resolution demonstrated a statistically significant difference from the initial measurements for both treatment groups ($P < 0.001$). However, there was no statistically significant difference in the defect fill (2.33 ± 1.36 mm vs. 1.91 ± 1.19 mm), % defect fill ($47.34 \pm 27.85\%$ vs. $46.28 \pm 26.51\%$) or % defect resolution ($46.01 \pm 27.56\%$ vs. $47.40 \pm 25.17\%$) between the groups.
Scheyer et al. (2002)	17 patients, 11 females, age 32–72 year	2, 2+3 walls	1. EMD + BDX (Bio-Oss) (test) 2. BDX alone (control)	6 months	There was no statistically significant difference for any of the measured clinical parameters. PD reduction for the EMD + BDX and BDX group was 4.2 ± 1.1 and 3.9 ± 1.3 mm, respectively ($P > 0.8$). Mean gain in CAL for the EMD + BDX and BDX groups was 3.8 ± 0.9 and 3.7 ± 1.5 mm, respectively ($P > 0.6$). Hard tissue measurements obtained at surgical reentry were used to calculate the bone fill (BF) and percent bone fill (%BF). The BF was 3.2 ± 1.4 and 3.0 ± 1.2 mm ($P > 0.6$) and the %BF was $63.3 \pm 16.3\%$ and $67.0 \pm 19.0\%$ ($P > 0.4$) for the EMD + BDX and BDX groups, respectively.
Sculean et al. (2002b)	24 patients, 14 females, age NA	1, 2, 3 walls	1. EMD + BDX (Bio-Oss) (test) 2. BDX alone (control)	12 months	At 1 year after therapy, the sites treated with EMD + BDX showed a reduction in PD from 10.0 ± 1.5 to 4.3 ± 1.4 mm and a CAL change from 10.9 ± 2.0 to 6.2 ± 1.9 mm ($P < 0.0001$). In the group treated with BDX, the PD was reduced from 9.7 ± 2.4 to 3.2 ± 0.7 mm and the CAL changed from 10.1 ± 2.3 to 5.2 ± 1.2 mm ($P < 0.0001$). Hard tissue fill was observed radiographically in all defects. Both treatments resulted in significant improvements of PD and CAL, but no statistically significant differences in any of the investigated parameters were observed between the test and control groups.

Sculean et al. (2002a)	28 patients, 15 females, age NA	1-2, 2, 3 walls	1. EMD + BG (Perioglas)(test) 2. BG alone (control)	12 months	At 1 year after therapy, the sites treated with EMD and BG showed a reduction in mean PD from 8.07 ± 1.14 to 3.92 ± 0.73 mm and a change in mean CAL from 9.64 ± 1.59 to 6.42 ± 1.08 mm ($P < 0.0001$). In the group treated with BG, the mean PD was reduced from 8.07 ± 1.32 to 3.85 ± 0.66 mm and the mean CAL changed from 9.78 ± 1.71 to 6.71 ± 1.89 mm ($P < 0.0001$). No statistically significant differences in any of the investigated parameters were observed between the test and control group. It was concluded that both therapies led to significant improvements of the investigated clinical parameters and the combination of enamel matrix derivative and bioactive glass does not seem to additionally improve the clinical outcome of the therapy.
<i>EMD + graft versus EMD</i>					
Guida et al. (2007)	27 patients, 14 women, aged 30-65 year	1-2 wall	1. EMD + ACBP (autogenous cortical bone particulate) group (14 defects) 2. EMD group (14 defects)	12 months	At 12 months, PD and CAL significantly improved from baseline in both groups ($P < 0.000$). No significant differences in terms of CAL gain and PD reduction were detected between groups (5.1 ± 1.7 vs. 5.6 ± 1.7 , respectively 4.6 ± 1.3 vs. 4.9 ± 1.8). However, defect distribution according to CAL gain was significantly different between groups (50% in test vs. 21% in controls) ($P < 0.05$). At 12 months, a significantly greater REC increase in the EMD group (1.1 ± 0.7 mm) compared to the EMD + ACBP group (0.3 ± 0.8 mm) was observed ($P < 0.05$). It was concluded that both EMD and EMD + ACBP treatments led to a significant improvement in clinical and radiographic parameters at follow-up with respect to presurgery condition. The combined approach resulted in reduced postsurgery recession and increased proportion of defects with substantial CAL gain (≥ 6 mm).
Yilmaz et al. (2010)	40 patients, 16 females, aged 30-50 year	2, 2 + 3 walls	1. EMD + AB (autogenous bone) 2. EMD	12 months	The test sites showed a reduction in the mean PD of 5.6 ± 0.9 mm ($P < 0.001$), a gain in the mean RAL of 4.2 ± 1.1 mm ($P < 0.001$) and a gain in the mean probing bone level (PBL) of 3.9 ± 1.0 mm ($P < 0.001$). The control group displayed a mean PD reduction of 4.6 ± 0.4 mm ($P < 0.001$), a mean RAL gain of 3.4 ± 0.8 mm ($P < 0.001$) and a mean PBL gain of 2.8 ± 0.8 mm ($P < 0.001$). RAL gains of ≥ 4 mm were measured in 90% of the test defects and in 55% of the controls. PBL gains of ≥ 4 mm were obtained in 85% of the test defects and in 25% of the control ones. The test treatment resulted in statistically higher PD reductions, RAL gains and PBL gains compared with the control ($P < 0.01$). It was concluded that, although the combination of EMD + AB resulted in statistically significant higher soft and hard tissue improvements 1 year after surgery compared with treatment with EMD, the clinical relevance of this finding is unclear.

(continued)

Table 3.6 (continued)

Author, year	Study population	Defect morphology	Treatment modalities	Study length	Outcomes
Gurinsky et al. (2004)	40 patients, 23 females, aged 19–76 year	1, 2, 3, 1–2, 2–3, 1–2–3 walled	1. EMD + DFDBA 2. EMD	12 months	The PD reduction for the combination therapy was $3.6 \text{ mm} \pm 0.2$, while the EMD alone had a probing depth reduction of $4.0 \text{ mm} \pm 0.3$. The CAL gain for the combined group was $3.0 \text{ mm} \pm 0.3$ and $3.2 \text{ mm} \pm 0.3$ for the EMD alone group. There was no significant difference between the treatment groups ($P > 0.10$). An evaluation of the amount of gingival recession that occurred after treatment for both groups revealed a mean increase in recession of $0.7 \text{ mm} \pm 0.2$ for the EMD alone group and $0.5 \text{ mm} \pm 0.3$ for the combination group. The amount of crestal resorption was greater for the EMD alone group ($0.9 \text{ mm} \pm 0.2$) than for the EMD + DFDBA group (0.1 ± 0.2) ($P < 0.04$). The mean value for bone fill for EMD + DFDBA group was $3.7 \text{ mm} \pm 0.2$, while the EMD alone demonstrated a mean bone fill of $2.6 \text{ mm} \pm 0.4$ ($P < 0.001$). These values correspond to 74.9% and 55.3% bone fill for the EMD + DFDBA and EMD alone, respectively ($P < 0.001$). The percent defect resolution for EMD + DFDBA was 75.7% and 69.2% for EMD alone, providing no statistically significant difference ($P > 0.10$) 90% bone fill when compared to EMD alone ($P < 0.001$). It was concluded that this study indicated that there may be an enhancement of hard tissue parameters when enamel matrix derivative is added to demineralized freeze-dried bone allograft.
Lekovic et al. (2000)	21 patients, 13 females, mean age 39 ± 1 year	2, 3 walls	1. EMD + BDX 2. EMD	6 months	Postsurgical measurements taken at 6 months revealed a significantly greater reduction in probing depth in the EMD + BDX group ($3.43 \pm 1.32 \text{ mm}$ on buccal sites and $3.36 \pm 1.35 \text{ mm}$ on lingual sites) when compared to the EMD group ($1.91 \pm 1.42 \text{ mm}$ on buccal sites and $1.85 \pm 1.38 \text{ mm}$ on lingual sites). The EMD + BDX group also presented with significantly more CAL gain ($3.13 \pm 1.41 \text{ mm}$ on buccal sites and $3.11 \pm 1.39 \text{ mm}$ on lingual sites) than the EMD group ($1.72 \pm 1.33 \text{ mm}$ on buccal sites and $1.75 \pm 1.37 \text{ mm}$ on lingual sites). Surgical reentry of the treated defects revealed a significantly greater amount of defect fill in favor of the EMD + BDX group ($3.82 \pm 1.43 \text{ mm}$ on buccal sites and $3.74 \pm 1.38 \text{ mm}$ on lingual sites) as compared to the EMD group ($1.33 \pm 1.17 \text{ mm}$ on buccal sites and $1.41 \pm 1.19 \text{ mm}$ on lingual sites). It was concluded that BDX has the ability to augment the effects of EMD in reducing PD, improving CAL and promoting defect fill when compared to presurgical levels.

Velasquez-Plata et al. (2002)	16 patients, 9 females; age, 36–65 year	2 + 3, 3 walls	1. EMD + BDX 2. EMD	8 months	Measures for PD reduction, attachment level gain, crestal resorption, % bone fill and % defect resolution did not present a statistically significant difference ($P > 0.10$). The most significant results were that gingival recession was greater for the group treated with EMD alone (0.8 ± 0.8 mm) compared to EMD + BDX (0.3 ± 0.6 mm) ($P = 0.04$) and amount bone fill was greater for EMD + BDX (4.0 ± 0.8 mm) compared to EMD alone (3.1 ± 1.0 mm) ($P = 0.02$). As a conclusion, the results demonstrated that a significant improvement in clinical parameters was observed. When comparing both modalities, a statistically significant difference was only found for gingival recession and bone fill, yielding a more favorable outcome towards the combined approach.
Zucchelli et al. (2003)	60 patients, 34 females; age, 34–62 year;	Angular bony defect (radiographic intrabony component >3 mm), CAL >6 mm	1. EMD + BDX 2. EMD	12 months	Both techniques resulted in clinically and statistically significant improvements between baseline and 1 year, in terms of CAL gain, PD reduction and radiographic bone fill; however, the EMD + BDX treatment showed statistically significantly greater CAL (5.8 ± 1.1 vs. 4.9 ± 1.0) and radiographic bone level gains (5.3 ± 1.1 vs. 4.3 ± 1.5), and less increase in gingival recession (0.4 ± 0.6 vs. 0.9 ± 0.5) than the EMD surgical procedure. It was concluded that the adjunctive use of EMD + BDX in grafting intrabony defects has the ability to improve clinical and radiographical outcomes achievable with EMP alone.
Sculean et al. (2005a)	30 patients, 16 females; age, 34–62 year;	1–2, 2, 3 walls	1. EMD + BG (Perioglas) 2. EMD	12 months	At 1 year after therapy, the EMD + BG group showed a reduction in mean PD from 8.5 ± 1.1 to 4.4 ± 1.2 mm ($P < 0.001$) and a change in mean CAL from 10.4 ± 1.5 to 7.1 ± 1.5 mm ($P < 0.001$). In EMD control group, the mean PD was reduced from 8.5 ± 1.5 to 4.0 ± 1.6 mm ($P < 0.001$) and the mean CAL changed from 10.2 ± 2.1 to 6.3 ± 2.2 mm ($P < 0.001$). In the test group, 12 sites (80%) gained at least 3 mm or more of CAL, whereas in the control group a CAL gain of 3 mm or more was measured at 13 sites (87%). No statistically significant differences in terms of PD reduction and CAL gain were observed between the two groups.
Sculean et al. (2007a)	30 patients, 16 females; age, 34–62 year same study group as above	1–2, 2, 3 walls	1. EMD + BG (Perioglas) 2. EMD	4 year	The test group demonstrated a mean CAL change from 10.3 ± 1.6 to 6.7 ± 1.2 mm ($P < 0.001$) and to 6.9 ± 1.0 mm ($P < 0.001$) at 1 and 4 years, respectively. No statistically significant differences were found between the 1- and 4-year results. The control group showed a mean CAL change from 10.4 ± 1.6 to 6.7 ± 1.1 mm ($P < 0.001$) at 1 year and 7.0 ± 0.9 mm ($P < 0.001$) at 4 years. No statistically significant differences between the two groups were found at 1 and 4 years.

(continued)

Table 3.6 (continued)

Author, year	Study population	Defect morphology	Treatment modalities	Study length	Outcomes
Bokan et al. (2006)	56 patients, 29 females; mean ages 56.6 ± 9.4 , 59.7 ± 7.6 , 55.0 ± 8.4 year	1–2, 2–3 walls	1. EMD + C (Cerasorb) 2. EMD 3. MWF	12 months	Treatment with EMD alone yielded a 3.9 ± 1.3 mm PD decrease and a 3.7 ± 1.0 mm CAL gain ($P < 0.001$), whereas EMD + C produced a 4.1 ± 1.2 mm PD reduction and a 4.0 ± 1.0 mm PAL gain ($P < 0.001$). These outcome parameters did not differ between the two groups. REC increased by 0.7 ± 1.3 mm. It was concluded that both EMD treatments showed similar clinical effects, with significant CAL gain.
Kuru et al. (2006)	23 patients with the mean age of 44.7 years	1, 2, walls defects with a PD ≥ 6 mm, radiographic angle about 45° , and intrabony defect depth ≥ 4 mm	1. EMD + BG (Perioglas) 2. EMD	8 months	At 8 months, the EMD + BG group showed a reduction of 5.73 ± 0.80 mm in PD ($P < 0.001$) and a change of 5.17 ± 0.85 mm ($P < 0.001$) in RAL. In the EMD gel group, the mean reduction of PD was 5.03 ± 0.89 mm ($P < 0.01$) while the mean RAL change was 4.06 ± 1.06 mm ($P < 0.01$). The intergroup comparison revealed a statistically significant difference both for PD ($P < 0.05$) and RAL measurements ($P < 0.05$). Greater PD reduction and attachment gain occurred with EMD + BG application. An evaluation of the hard tissue findings indicated that both treatment modalities result in bone gain at 8 months. The EMD + BG group showed 2.76 ± 0.69 mm ($P < 0.001$) of RBL change considered as the radiographic bone gain, while this was 2.15 ± 0.42 mm ($P < 0.01$) for the EMD group. The difference between the groups was in favor of the combined group ($P < 0.05$).
Jepsen et al. (2008)	73 patients, 23 females; age 18–70 year	1, 2, 1–2 wall, circumferential	1. EMD + SBC 2. EMD	6 months	Both treatment modalities led to significant clinical improvements. Change in bone fill 6 months after surgery was 2.0 ± 2.1 mm in the test group and 2.1 ± 1.2 mm in the control group. A gain in CAL of 1.3 ± 1.8 mm in the test group and 1.8 ± 1.6 mm in the control group was observed. PD change in the two groups was 1.93 ± 1.8 mm for EMD + SBC and 2.55 ± 1.8 mm for EMD. One week after surgery, primary closure was maintained in 95% of the test sites and 100% of the control sites. No differences in patients' perceptions were found. It was concluded that similar clinical outcomes following both treatment modalities were obtained.

RAL relative attachment level, BG bioactive glass, DFDBA demineralized freeze-dried bone allograft, PD probing depth, SBC synthetic bone graft (Straumann Bone Ceramic, Straumann, Basel, Switzerland). SBC is a synthetic bone graft substitute of medical-grade purity in particulate form composed of biphasic calcium phosphate – a mixture of 60% hydroxylapatite, and of 40% of the beta form of tricalcium phosphate, RBL radiographic bone level

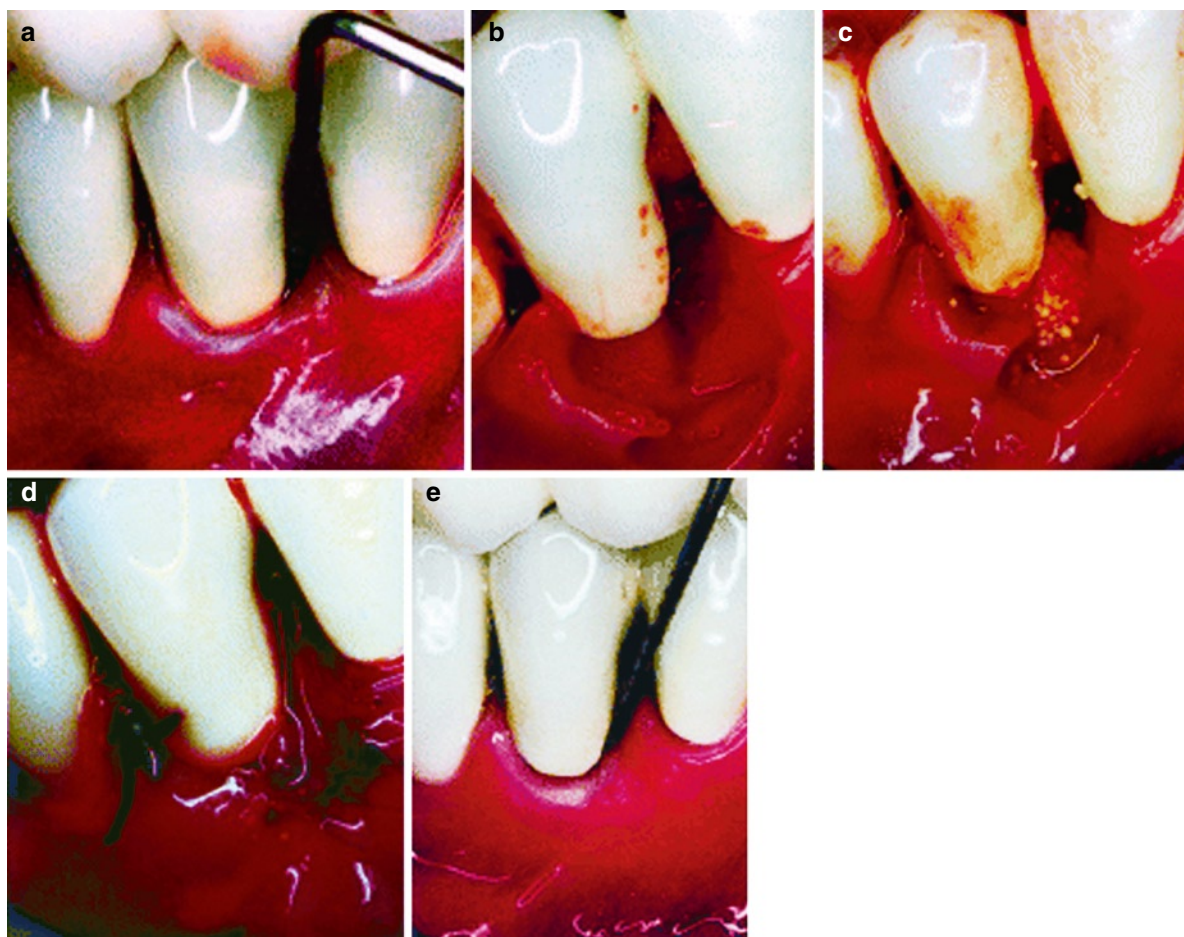


Fig. 3.7 Microsurgical access flap for buccally located defects. (a) Site treated with Emdogain® in combination with Cerasorb® and microsurgically modified “papilla-preservation” technique, with 7 mm loss of attachment before surgery; (b) flap deflection with buccal one+two-wall bony defect; (c) application of

Emdogain® and Cerasorb®; (d) flap reposition and fixation with microsuture (Vicryl 8/0 Ethicon, needle GS-9); and (e) situation 1 year after surgery with unaltered gingival contour in comparison with the preoperative situation (Bokan et al. 2006. Reprinted with permission from John Wiley & Sons)

effects. EMD in conjunction with bovine bone grafts showed 0.78 mm (95% HPD intervals: 0.15, 1.37) greater PD reduction, while other bone grafting materials did not show significantly additional treatment effects. EMD in conjunction with bovine bone grafts showed 0.78 mm (95% HPD intervals: 0.15, 1.37) greater PD reduction, while other bone grafting materials did not show significantly additional treatment effects. EMD in conjunction with bovine bone grafts and a resorbable membrane (Bio-Gide, Osteohealth, Shirley, NY, USA) seemed to achieve the greatest treatment effects (1.28 mm greater than EMD alone, 95% HPD intervals: -0.71, 3.21), but the confidence intervals were large. EMD in conjunction with bovine bone grafts showed 0.93 mm (95% HPD intervals:

0.48, 1.54) greater CAL gain than EMD alone, and EMD in conjunction with Perioglas showed 0.54 mm (95% HPD intervals: -0.03, 2.13) greater gain than EMD alone. However, in the adjusted analysis, Perioglas did not show an additional benefit (Fig. 3.9) (Tu et al. 2010).

3.5.3 Hard Tissue Response After Emdogain Treatment of Infrabony Pockets

Several systematic reviews were focused on the question, if and to what extent EMD promotes the

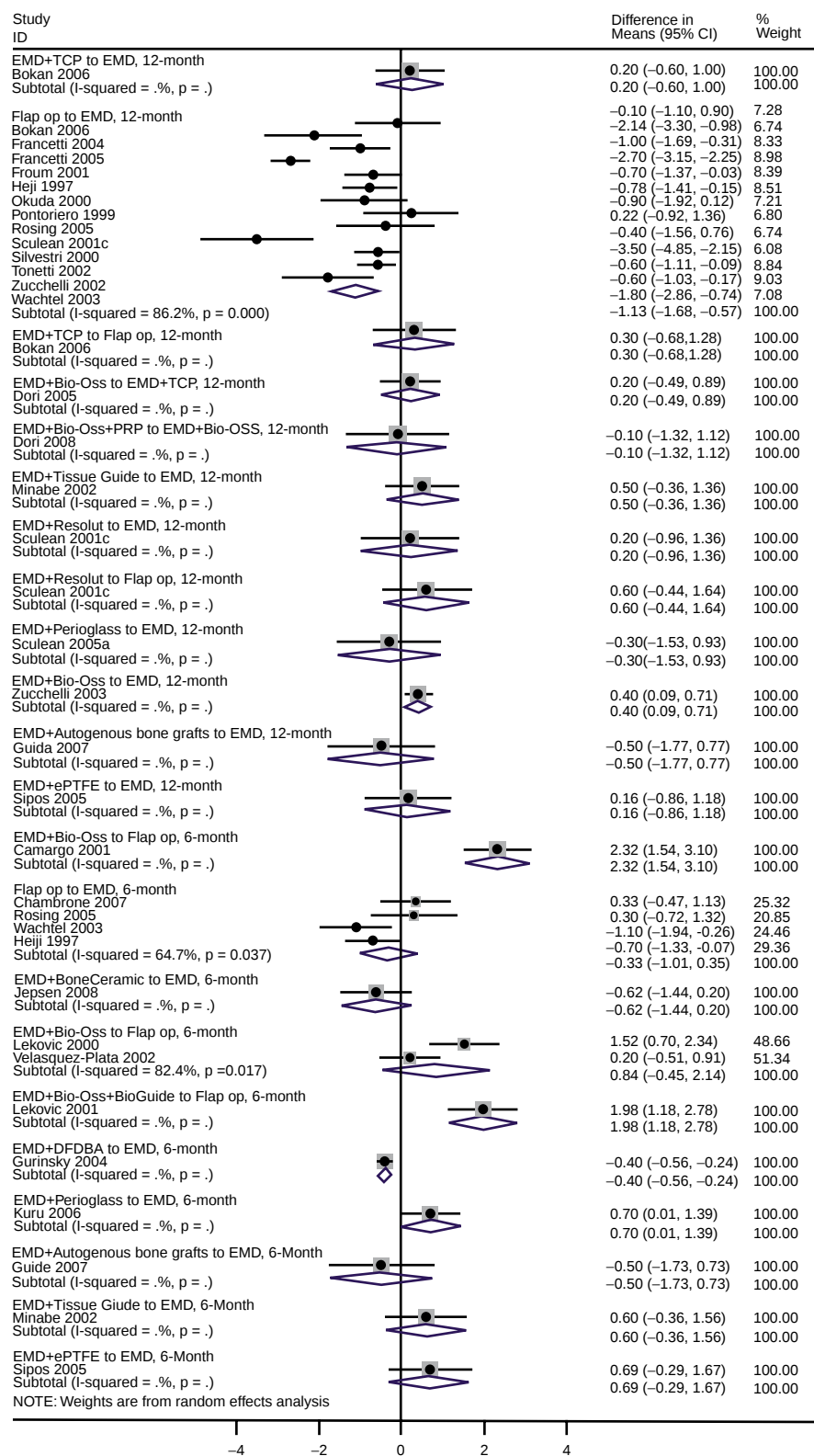


Fig. 3.8 Forest plot of the standard pair-wise meta-analysis for probing pocket depth reduction when different types of bone grafts were treated as separate groups and different types of

barrier membranes were treated as separate groups (Tu et al. 2010. Reprinted with permission from John Wiley & Sons)

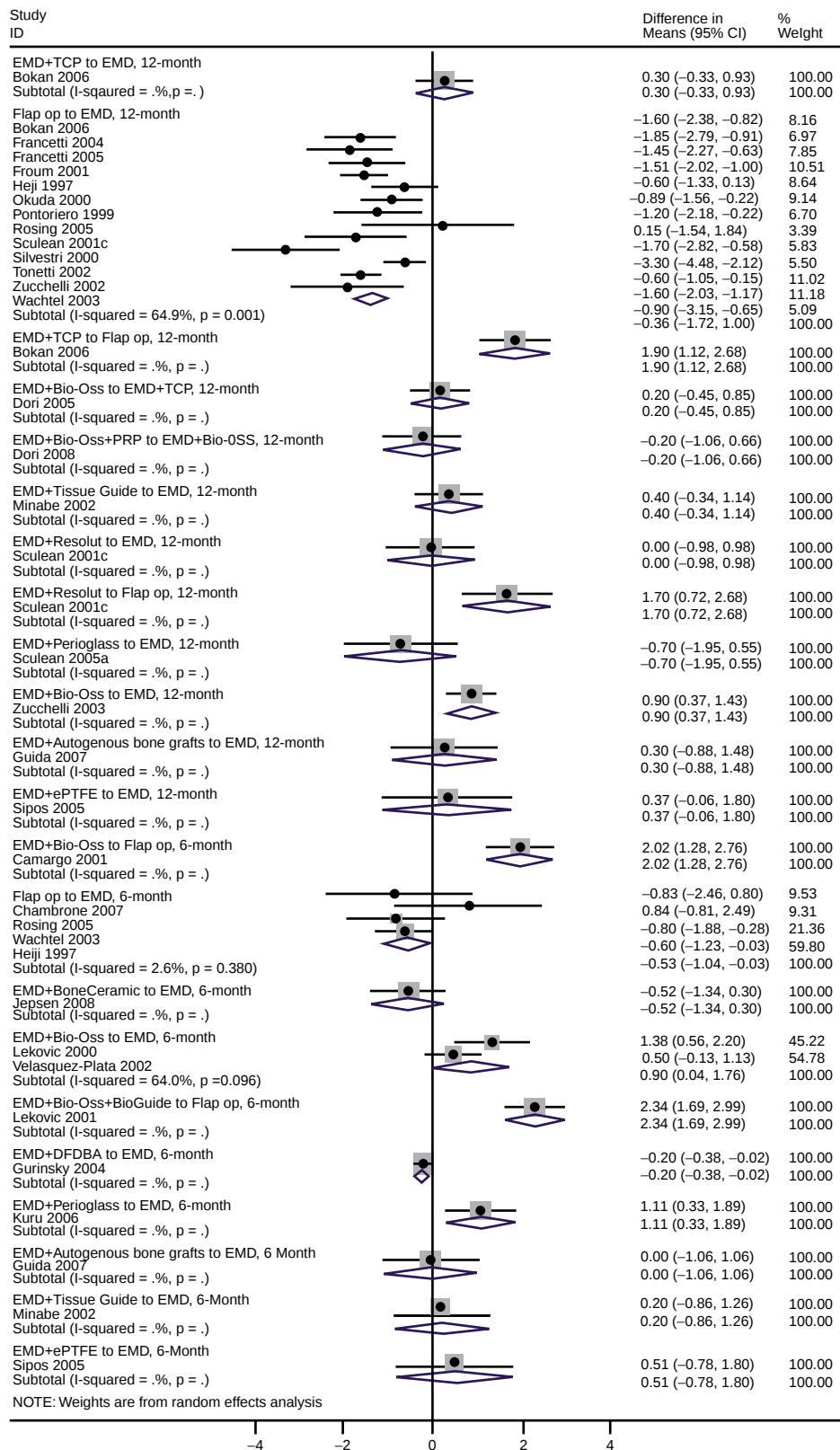


Fig. 3.9 Forest plot of the standard pair-wise meta-analysis for clinical attachment level gain when different types of bone grafts were treated as separate groups and different types of barrier

membranes were treated as separate groups (Tu et al. 2010. Reprinted with permission from John Wiley & Sons)

regeneration of bone (Sculean et al. 2007c; Rathe et al. 2009; Tu et al. 2010; Kalpidis and Ruben 2002).

Animal studies suggest that EMD seems to lead to more bone regeneration of infrabony defects compared to OFD; however, EMD application might result in more bone formation when applied in supporting defects compared to nonsupporting defects. Further, most animal studies suggest that combined therapy (GTR + EMD) of infrabony defects might not lead to better results than GTR therapy alone (Rathe et al. 2009).

Unfortunately, none of the histomorphometric studies reporting on human data compared the amount of bone regeneration in periodontal defects after the use of EMD with a control surgery (i.e., OFD and coronal advanced flap). If bone regrowth was evaluated after the use of EMD and compared with results obtained after utilizing the GTR therapy with resorbable membranes, GTR therapy resulted in a higher degree of bone regeneration than treatment with EMD (Rathe et al. 2009).

In 5 out of 13 studies evaluated by Rathe et al. (2009), no new bone formation could be evaluated histologically after EMD treatment; further, bone regeneration was minimal (0.2–0.5 mm) in four further defects. In the remaining four defects of the EMD group, bone regeneration could be evaluated with a range of 1.8–2.2 mm. Infrabony defects treated with GTR showed at least minimal bone formation (0.1 and 0.2 mm) in two defects, whereas the 12 remaining defects showed bone regrowth within a range of 1–3 mm (Rathe et al. 2009).

For infrabony defect fill, network meta-analysis showed that EMD alone achieved 3.10 mm at 12 months, which was 1.53 mm (95% HPD intervals: 0.91, 2.13) greater than that for flap operation. EMD in conjunction with bone graft achieved 1.10 mm (95% HPD intervals: 0.56, 1.82) greater defect fill, while EMD in conjunction with GTR showed no additional treatment effects. The adjustment of baseline CAL has only a small impact on the estimated treatment effects between combination therapies and EMD alone. Studies using the split-mouth design seemed to report less favorable results than those using the parallel-group design. Results from standard meta-analysis showed that EMD alone achieved significantly greater defect fill than flap operation at 12 months, while EMD in conjunction with bone grafts achieved a small additional defect fill than EMD alone (Fig. 3.10).

3.5.4 Factors That Determine Emdogain Outcomes in the Treatment of Infrabony Defects

The differences in the CAL gain reported in different studies might be explained by several factors related to design and treatment procedures: differences in patient population, baseline characteristics of the periodontal defects, measurement techniques, surgical approach, operator skill with a given material, type of defect treated, levels of plaque control, etc. (Parashis and Tsiklakis 2000).

Age was found to have no influence on CAL gain or radiographic bone gain (Bratthall et al. 2001). One study that evaluated whether *gender* has any effect found no statistically significant differences in CAL gain between males and females (Parodi et al. 2000).

In the study performed by Heijl et al. (1997), no effort was made to exclude any patients with smoking habits, various *systemic disorders*, etc. Furthermore, patients included in that clinical trial had been subjected to systematic periodontal treatment including repeated mechanical debridement and therapy supplemented with antimicrobial as well as surgical procedures in the experimental areas. The overall assessment of these patients is that they presented with a periodontal disease that was not responsive to conservative periodontal therapy.

A high *level of plaque control* and the frequency of professional maintenance are directly correlated with CAL gain and bone fill. Heden et al. (1999) observed that in his study the mean amount of probing attachment gain (4.6 mm) achieved was somewhat greater than that reported by Heijl et al. (1997) and Pontoriero et al. (1999). This additional improvement of the clinical attachment level was not related to different baseline characteristics of the defects treated, but was the result of a more stringent plaque control regime used both before and after regenerative surgery used in the trial. First of all, all patients had been treated for advanced periodontal disease and were included in a carefully supervised supportive periodontal care program. Furthermore, during the first 6 postoperative weeks, the patients rinsed, 2× daily, with 0.1–0.2% chlorhexidine digluconate in an attempt to reduce further the number of oral bacteria. During this initial 6-week interval, no mechanical instrumentation of the surgical site was allowed and only subsequently was the self-performed toothbrushing and the interdental

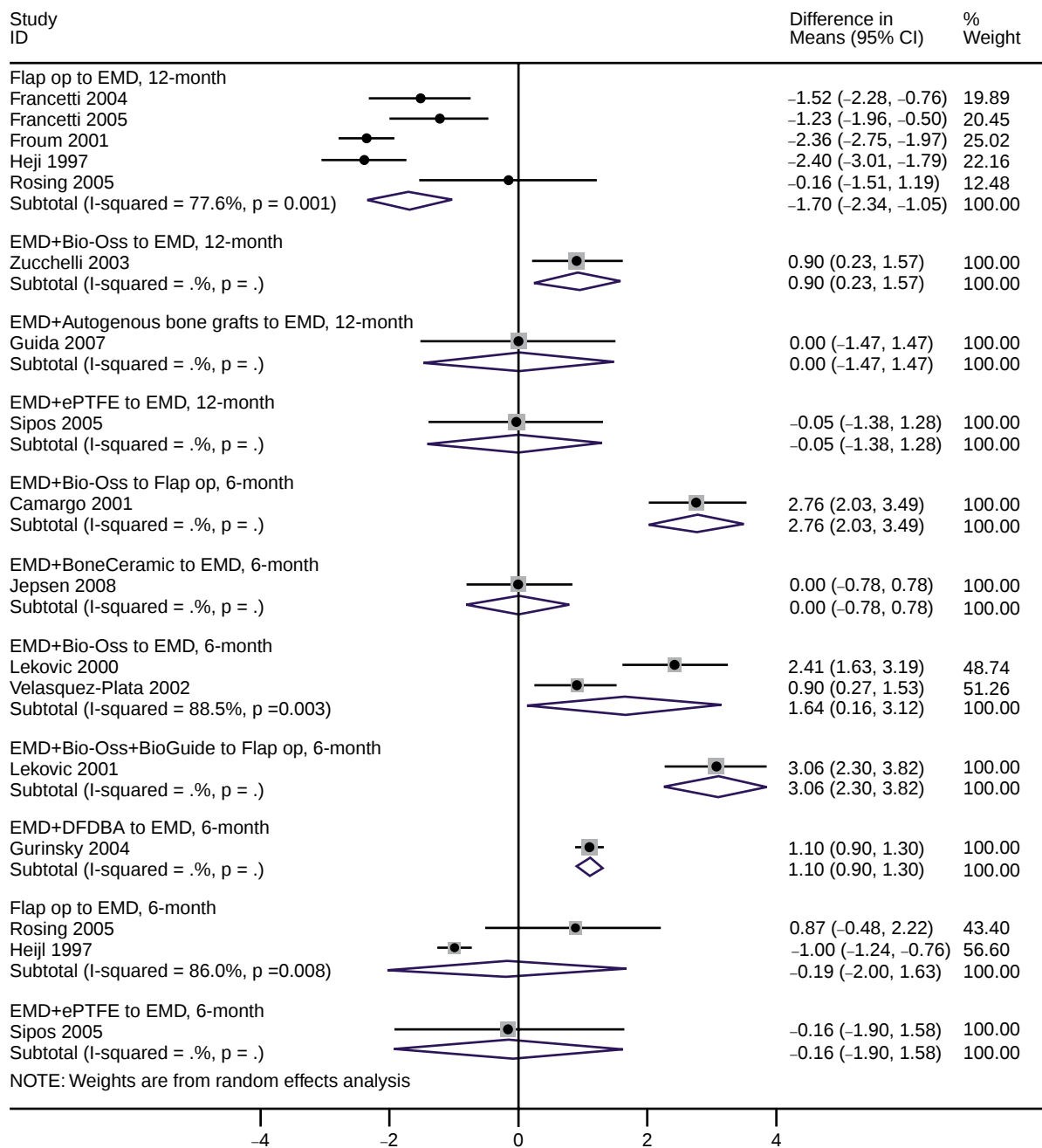


Fig. 3.10 Forest plot of the standard pair-wise meta-analysis for infrabony defect fill when different types of bone grafts were treated as separate groups and different types of barrier mem-

branes were treated as separate groups (Tu et al. 2010. Reprinted with permission from John Wiley & Sons)

cleaning with mechanical aids recommended and professional cleaning reinstituted. There are reasons to suggest that the reduced bacterial load and the exclusive use of chemical plaque control measures may have established optimal conditions for healing at the treated

sites (Heden et al. 1999). In contrast, plaque accumulation was not found to be a determining factor for CAL gain (Tonetti et al. 2002), although it should be noted that the plaque scores in this study were very low and had a small standard deviation.

Smoking status was showed to be a negative predictor for both EMD regenerative procedures. Although some investigators did not detect significant differences in CAL gain according to smoking status (Parodi et al. 2000; Sculean et al. 2002a; Trombelli et al. 2002a), there is also ample evidence that better treatment outcomes were found for nonsmokers than for smokers (Heijl et al. 1997; Heden et al. 1999; Heden 2000; Bratthall et al. 2001; Tonetti et al. 2002; Zucchelli et al. 2002; Leknes et al. 2009). A negative correlation between smoking with radiographic bone regeneration has also been reported (Bratthall et al. 2001).

Conflicting results were reported when the influence of the *anatomic location of treatment (mandible or maxilla)* on the results obtained following treatment with EMD was assessed (Heijl et al. 1997; Bratthall et al. 2001).

Clinical studies have demonstrated that the CAL gain following conventional and regenerative periodontal surgery is strongly dependent on the *initial defect depth*, i.e., the greater the initial defect depth, the greater the PD reduction and the CAL gain (Sculean et al. 1999a; Okuda et al. 2000; Zetterström et al. 1997; Heden et al. 1999; Pontoriero et al. 1999; Parodi et al. 2000; Bratthall et al. 2001; Tonetti et al. 2002; Zucchelli et al. 2002; Silvestri et al. 2003). Baseline PD and radiographic defect depth was also positively correlated to radiographic bone gain (Bratthall et al. 2001). However, Yukna and Mellonig (2000) reported no relationship between defect depth and histologic results.

The number of osseous walls and width of the defect (angle of the defect) have been shown to correlate with the osseous fill (Froum et al. 2001). Thus in the studies of Heden et al. (1999), Heijl et al. (1997) and Zetterström et al. (1997) the majority (94%) of the defects were one- and two-wall character, while in the study of Parashis and Tsiklakis (2000) and Sculean et al. (1999a) the defects were a combination of two and three walls, which sometimes have exceptionally good osseous regrowth after flap surgery alone (Polson and Heijl 1978). Intrabony defects with three walls had a 269% higher chance than one-wall intrabony defects to gain 3 mm CAL or more (Tonetti et al. 2002). However, several studies could not demonstrate a correlation between the number of defect walls and the regenerative success with EMD (Heden 2000; Bratthall et al. 2001; Minabe et al. 2002, Tsitoura et al. 2004). Among the considered defect characteristics, markedly corticalized and very cancellous bleeding intrabony defects had significantly lower CAL gains than defects with a regular cribriform bony lining. Defects

with either dense cortical or very cancellous bleeding walls displayed an 86% reduced chance over a normally corticalized, cribriform defect (Tonetti et al. 2002).

It was disclosed that soft tissue *recession* has a strong influence on the gain of probing attachment (Heden et al. 1999). From several studies this recession amounted from 0.5 to 0.8 mm (Parashis and Tsiklakis 2000; Heden et al. 1999; Froum et al. 2001) in contrast with the soft tissue shrinkage reported by Sculean et al. (1999a), Parodi et al. (2000), and Pontoriero et al. (1999).

CAL gain was significantly influenced by the amount of *presurgical interdental supracrestal soft tissue* (Trombelli et al. 2002a). Thirty-five consecutively treated osseous defects were analyzed 9–12 months after surgery. Clinical attachment level gain and depth of the defect (calculated as the vertical distance in mm from the bone crest and the most apical extension of the defect where the periodontal ligament space was considered as having a normal width) gain averaged 4.7 and 3.9 mm, respectively. A new variable, representing a surrogate measurement of the amount of supracrestal soft tissue (SUPRA), was created using the following equation: $SUPRA = (PBL - IBD) - REC$ (in mm), where PBL = probing bone level distance from the cemento-enamel junction to the bottom of the defect, IBD = intrabony component of the defect: distance from the most coronal extension of the interproximal bone crest to the bottom of the defect REC = gingival recession. Clinical attachment level gain was significantly influenced by the amount of interdental supracrestal tissues (SUPRA). Presence of thick interdental tissues may have facilitated flap management and suturing technique while maximizing the possibility to achieve primary closure in the interproximal area. It was also speculated that a surgical procedure aimed at supracrestal soft tissue preservation may restrict the detrimental effect of smoking on postsurgery healing dynamics and tissue remodeling (Trombelli et al. 2002a). Periosteal incisions did not influence the treatment outcomes (Tonetti et al. 2002).

Several studies (Heden et al. 1999; Heden 2000; Zucchelli et al. 2002; Silvestri et al. 2003) have revealed that *bleeding on probing* during follow-up examinations has an adverse effect on the treatment outcomes.

It is also implied that the outcome of Emdogain treatment of intrabony defects is as *operator sensitive* as GTR procedures (Heden et al. 1999).

Traditionally such root conditioners were used to chemically modify the root surface in order to stimulate periodontal regeneration. Various “conditioning agents” have been used and the manufacturer of EMD produces one root conditioner called PrefGel composed of 24% ethylenediaminetetra-acetic acid (EDTA) at neutral pH. Several studies (Sculean et al. 2006; Parashis et al. 2006) and systematic reviews (Mariotti 2003; Esposito et al. 2009) failed to show the efficacy of such procedures.

Systemic administration of antimicrobials (amoxicillin and metronidazole) following surgical placement of EMD did not produce statistically superior probing depth reduction or CAL gain compared with treatment with EMD alone (Sculean et al. 2001d). Similarly, the use of *nonsteroidal anti-inflammatory drugs* (COX-2 inhibitors) following regenerative periodontal surgery with EMD did not result in additional clinical improvements when compared with treatment with EMD alone (Sculean et al. 2003e).

3.6 Summary of Studies Evaluating the Efficacy of Emdogain in the Treatment of Furcation Lesions

Furcation defects have presented a major challenge to therapists because of their unique anatomical characteristics and their variable response to treatment. Resective and tissue attachment techniques have slowed the progression of disease but have been unable to stop attachment loss in most longitudinal studies. Consequently, furcation involvement has been synonymous with more advanced forms of periodontitis and a more guarded prognosis for long-term tooth retention (McClain and Schallhorn 2000).

In animals, EMD seems to result in more bone formation compared to OFD in both furcation class II and III defects, but treatment of class III furcation defects with EMD is unpredictable. Additionally, there seems to be no additional benefit of combined therapy (GTR + EMD) in furcation defects (Rathe et al. 2009).

Few controlled clinical studies have investigated the treatment of furcation defects using flap surgery with and without EMD.

A multicenter, randomized controlled, split-mouth clinical study compared enamel matrix derivative (EMD; test) with barrier membranes (control) for the treatment of mandibular buccal class II furcation

defects (Jepsen et al. 2004; Meyle et al. 2004; Hoffmann et al. 2006). Forty-five patients with 90 comparable defects on contralateral molars were included. Defects were randomly assigned to EMD or bioabsorbable barrier membrane; the contralateral defect received the alternative treatment. Assessments at baseline and 8 and 14 months included gingival margin levels, probing depths, bleeding on probing, vertical attachment levels and vertical bone sounding from a stent at five buccal sites/teeth. The results revealed that both treatment modalities led to significant clinical improvements. The median reduction of open horizontal furcation depth was 2.8 mm with the corresponding interquartile interval (1.5 mm, 3.5 mm) at test sites compared with 1.8 mm (1.0 mm, 2.8 mm) at control sites. The mean reduction was 2.6 ± 1.8 mm at test sites compared with 1.9 ± 1.4 mm at control sites. The frequency of complete furcation closure was 8/45 (test) and 3/45 (control); partial closure, 27/45 in both groups; no change, 9/45 and 11/45, respectively; and deterioration, 1/45 and 4/45, respectively. The frequency of no pain or no swelling at 1 week postsurgery was 62% and 44%, respectively, at the test sites and 12% and 6% at the control sites (Jepsen et al. 2004). However, there was slightly more recession in the mid-furcation site following membrane treatment ($P = 0.04$). There was no measurable bone resorption in EMD sites, whereas a slight resorption occurred with membrane treatment. Furcation morphology at the time of surgery was not associated with clinical outcome, irrespective of the treatment (Meyle et al. 2004). Furthermore, it also revealed a slight superiority of regenerative furcation therapy using enamel matrix derivative when compared with membranes in the older age group, in nonsmokers, in male patients and in patients with poor hygiene (Hoffmann et al. 2006). The study concluded that there was a significantly greater reduction in horizontal furcation depth and a comparatively lower incidence of postoperative pain/swelling following enamel matrix derivative compared to membrane therapy.

Chitsazi et al. (2007) evaluated the efficacy of open flap debridement (OFD) with and without enamel matrix derivatives (EMD) in the management of buccal class II furcation involvement. Twenty similar bilateral class II furcation defects in ten healthy nonsmoker patients were selected. One defect in each subject was treated with OFD alone (OFD group) and the contralateral one with OFD and simultaneous application of enamel matrix derivatives (EMD

group). Clinical probing depth, vertical clinical attachment level, horizontal clinical attachment level and the location of the gingival margin, horizontal probing depth of bony defect (E-HPD), vertical depth of bone crest, vertical depth of the base of bony defect (V-DBD) and length of the intrabony defect were measured at baseline and during reentry surgery after 6 months. Vertical decrease in the CAL in the EMD and OFD groups were 1.45 and 0.9 mm, respectively. The postoperative gingival recession was not significant in both groups ($P > 0.05$). Moreover, the probing depth reduced by 1.95 mm in the EMD site and by 0.9 mm in the OFD site, suggesting that the gain in attachment is the major contributor to the reduction in the probing depth. Horizontal attachment gain in the EMD and OFD groups were 1.9 mm and 0.6 mm, respectively. Both within-group and between-group differences were significant. Through the surgical approach (reentry surgery), the formation of bone was evaluated. The amount of bone formation as evidenced by filling of the defect with new bone was 1.25 mm in the EMD site and 0.85 mm in the OFD site. Regarding the mean primary depth of the defects in the EMD group (2 mm), 62.5% of the defects were repaired with bone formation. Crestal bone resorption equaled 0.4 mm in the EMD site and 0.35 mm in the OFD site. The intrabony component of the defect decreased by an average of 1.65 and 1.2 mm in the EMD and the OFD groups, respectively. Both the within-group and the between-group differences at 6 months postoperatively were significant. Because the amount of crestal bone resorption was minimal, these changes mostly reflected the filling of the intrabony defect. HPD of the defect with open access examination showed a reduction of 2 mm in the EMD and 0.8 mm in OFD groups. This fact was suggestive of the efficiency of EMD in the resolution of the horizontal components of the furcation defects. Based on the reported results, it was concluded that both treatment protocols were effective in enhancement of the clinical parameters of the soft tissue healing. However, EMD application resulted in a more efficient healing of the periodontal hard tissues with reference to the vertical and horizontal defect resolution.

Proximal furcation involvements represent a challenge to periodontal therapy with unpredictable results obtained with the GTR therapy due to the difficulty to access, view and debride the furcation area, as well as membrane adaptation (Metzler et al. 1991, Mellonig

et al. 1994; Pontoriero and Lindhe 1995; Rosen et al. 1997; Avera et al. 1998; Machtei 2001). Recently, a double blind randomized prospective controlled clinical parallel study compared the clinical outcomes after OFD+24% EDTA root conditioning (control group, $n=15$) with OFD+24% EDTA conditioning+EMD protein application (test group, $n=15$) (Casarin et al. 2008). Plaque index, bleeding on probing, probing depth (PD), gingival margin position, relative vertical and horizontal clinical attachment level, vertical and horizontal bone level and furcation closure were evaluated immediately before and 2, 4 and 6 months after the surgeries. Re-assessment visits occurred every 15 days during the first month and monthly until the sixth month. During these appointments, the examiner recorded the clinical periodontal parameters and checked any change in the medical or health status. At the end of the appointment, a session of supragingival prophylaxis was performed as necessary. There were no differences between the groups with regard to gingival recession and reduction in PD ($P > 0.05$). In both groups, a significant improvement in relative horizontal clinical attachment level ($P < 0.05$) was obtained. The gain in relative horizontal clinical attachment level was 1.21 ± 2.28 mm for the control group and 1.36 ± 1.26 mm for the test group ($P < 0.05$). The relative vertical clinical attachment level gain was 0.39 ± 1.00 and 0.54 ± 0.95 mm for the control and test groups, respectively ($P > 0.05$). At baseline and 6 months, the furcation sites were anesthetized and bone probing was performed to determine the bone level. There was an improvement in both the vertical and horizontal bone level at 6 months ($P < 0.05$). The gain in horizontal bone level in the control group was 1.00 ± 1.79 mm, whereas in the test group the gain was 1.17 ± 1.38 mm. The vertical bone level gain was 1.04 ± 1.12 and 0.82 ± 1.82 mm for the control and test groups, respectively. There was no difference between the groups for the vertical and horizontal bone level. In the control group, 67% of the proximal furcations maintained a class-II diagnosis, while in the test group only 33% still received this classification ($P = 0.01$). Similarly, the number of class-I furcation was significantly higher in the test than in the control group ($P = 0.05$). The evaluation of the closed furcations demonstrated that, in the test group, two proximal furcations were not detectable by clinical examination. In contrast, the control group did not demonstrate any closed furcation. In conclusion, this controlled RCT

showed that the application of EMD in proximal furcations did not promote a superior reduction in PD or gain in the clinical and osseous attachment level, but allowed a higher rate of conversion of class-II to class-I furcations.

3.7 Summary of Studies Evaluating the Efficacy of Emdogain in the Treatment of Supra-alveolar-type Defects

Only two trials examined the efficacy of EMD in the treatment of suprabony lesions adding valuable information for the clinician in decision making regarding effective treatment alternatives for various types of periodontal destruction (Yilmaz et al. 2003).

Yilmaz et al. (2003) assessed the clinical and radiographic outcome of horizontal type of bone loss over a period of 8 months following periodontal surgery with adjunctive use of EMPs. Twenty patients, who received nonsurgical periodontal therapy and had radiographic horizontal bone loss with an associated probing depth (PD) of ≥ 4 mm at the maxillary incisor/canine segment, were included. One side of the selected segment divided by the midsagittal plane was treated with EMP as part of a crevicular flap. The other side was treated either with a similar intracrevicular or a reverse bevel incision as part of a conventional flap debridement. Therefore, patients were divided into two groups: (1) Ten patients treated with Emdogain (test) (T1) and flap debridement with an intracrevicular incision (control 1) (C1) and (2) Ten patients treated with Emdogain s (test) (T2) and flap debridement with a reverse bevel incision (control 2) (C2). Clinical improvements with EMP application, especially at sites with deep pockets (4–6 mm), were found to be superior to conventional procedures in terms of PD reduction (2.87–2.92 mm in the T1-T2 groups versus 1.53–2.87 mm in the C1-C2 groups), attachment gain (2.16–2.27 mm versus 0.54–1.56 mm) and recession (0.68–0.67 mm versus 1.06–1.35 mm). Treatment of 1–3 mm pockets by the conventional flap performed with both incisions resulted in a tendency for loss of attachment. Adjunctive EMP application maintained attachment levels in the shallow pockets. More gingival recession occurred in controls than the EMP-treated groups at both shallow and deep periodontal pockets (Yilmaz et al. 2003) (Fig. 3.11).

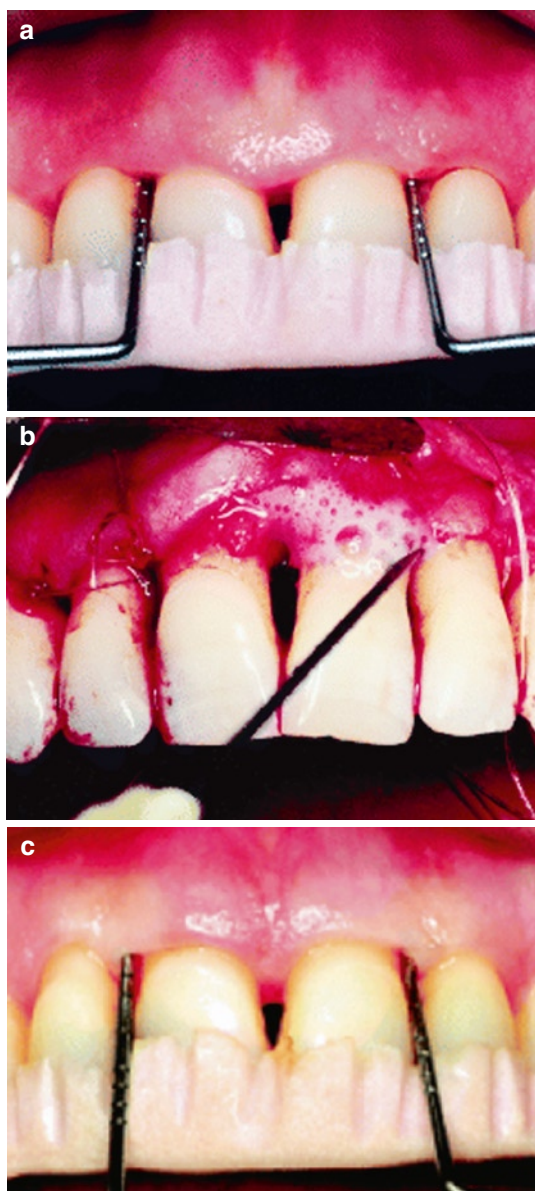


Fig. 3.11 Enamel matrix proteins (EMPs) in the treatment of periodontal sites with horizontal type of bone loss. (a) Preoperative clinical view. (b) Application of Emdogain® gel. (c) Eight-month postoperative clinical view (Yilmaz et al. 2003. Reprinted with permission from John Wiley & Sons)

The aim of a recent pilot study, performed by Jentsch and Purschwitz (2008), was to check if the results of access flap surgery in suprabony defects are improved by additional application of EMD. A total of 70 teeth were treated with EMD; 28 teeth were treated by access flap. A significant reduction in PD occurred in the test group (1.55 mm after

12 months for overall measurements while in the control group a difference of 0.41 mm was measured). These results are significantly different ($P < 0.001$). The overall changes of the CAL were significant only in the test group ($P < 0.001$). Here, the improvement after 12 months is 0.97 mm in the test group and 0.07 mm in the control group. These changes were significantly different between both groups ($P < 0.001$). No significant difference between the groups could be found for BOP. At initially higher PDs, a greater benefit of the use of enamel matrix protein derivative (EMD) with respect to PD reduction and attachment gain was seen. The improvement was 1.78 mm for PD and 1.20 mm for attachment level in the test group for sites with an initial PD of 4–6 mm. The corresponding results for sites ≥ 7 mm were 4.19 and 2.72 mm. The results for PD reduction and attachment gain were significantly better in the test group than in the control group. In the control group, the PD reduction were 0.61 and 1.95 mm and the attachment gain 0.36 and 0.78 mm, respectively. The differences between both groups were always significant for PD reduction and attachment level gain ($P < 0.001$ and $P = 0.009$). The data suggested a significant clinical benefit of supplementary application of EMD during surgical treatment of periodontitis of supra-alveolar pockets, especially in deeper pockets.

3.8 Summary of Studies Evaluating the Efficacy of EMD in the Treatment of Gingival Recession

Another indication for EMD is the gingival recession, frequently associated with dental hypersensitivity and/or lack of esthetics of an exposed root. Animal and human histological studies have revealed that treatment of buccal recessions with coronally positioned flap and EMD can result not only in a covering of the gingival recession but also in the formation of the cementum, periodontal ligament and bone (Sculean et al. 2007c). Several controlled clinical studies (Table 3.7) summarized by three meta-analyses (Table 3.8) have addressed the issue of the efficacy of enamel matrix derivative in the treatment of gingival recession. Berlucchi et al. (2005) suggested that baseline recession depth and flap thickness may influence the outcome of marginal tissue recession therapy

with coronally advanced flap (CAF) plus EMD at 12 months.

Cheng et al. (2007) reviewed relevant studies and performed a meta-analysis of coronally advanced flap (CAF), coronally positioned flap + chemical root surface conditioning (CAF + RC) (Amarante et al. 2000; Hägewald et al. 2002; Modica et al. 2000; Woodyard et al. 2004) or coronally positioned flap + enamel matrix derivative (CAF + EMD) (Abbas et al. 2003; Berlucchi et al. 2002, 2005; Hägewald et al. 2002; McGuire and Nunn 2003; Modica et al. 2000; Nemcovsky et al. 2004) for the treatment of Miller class I and II gingival recession. All three groups achieved considerable root coverage and gains in clinical attachment and maintained the amount of keratinized tissue and shallow probing pocket depths. At 12 months, the mean gains in clinical attachment level were 1.69 ± 0.15 mm (χ^2 for heterogeneity: 2.613 ± 1 , $P = 0.106$) in the coronally positioned flap group, 3.10 ± 0.00 mm in the coronally positioned flap + chemical root surface conditioning group and 3.61 ± 0.50 mm (χ^2 for heterogeneity: 24.303 ± 3 , $P < 0.05$) in the coronally positioned flap + EMD group. At 12 months, the mean gains in keratinized tissue were 0.10 ± 0.41 mm (χ^2 for heterogeneity: 2.613 ± 1 , $P = 0.106$) in the coronally positioned flap group, 0.30 ± 0.00 mm in the coronally positioned flap + chemical root surface conditioning group and 0.61 ± 0.14 mm (χ^2 for heterogeneity: 53.647 ± 2 , $P < 0.05$) in the coronally positioned flap + EMD group. The gingival recession depth in the coronally positioned flap + EMD group decreased from 3.91 ± 0.35 to 0.62 ± 0.36 mm (χ^2 for heterogeneity: 21.537 ± 4 , $P < 0.05$) at 6 months and decreased from 3.91 ± 0.42 to 0.72 ± 0.40 mm (χ^2 for heterogeneity: 24.739 ± 4 , $P < 0.05$) at 12 months. In the coronally positioned flap group, the gingival recession depth decreased from 3.36 ± 0.36 to 0.80 ± 0.42 mm (χ^2 for heterogeneity: 76.469 ± 5 , $P < 0.05$) at 6 months and decreased from 3.18 ± 0.57 to 1.37 ± 0.04 mm (χ^2 for heterogeneity: 2.613 ± 1 , $P = 0.106$) at 12 months. In the coronally positioned flap + chemical root surface conditioning group, gingival recession depth decreased from 3.62 ± 0.29 to 1.17 ± 0.25 mm (χ^2 for heterogeneity: 27.290 ± 3 , $P < 0.05$) at 6 months and decreased from 3.90 ± 0.00 to 1.00 ± 0.00 mm at 12 months. The results in the coronally positioned flap group were $74.12 \pm 15.80\%$ root coverage percentage at 6 months and $54.16 \pm 0.00\%$ root coverage percentage at 12 months. In the coronally positioned flap + chemical root surface conditioning group, the results were

Table 3.7 Characteristics of the controlled clinical studies with the adjunctive use of enamel matrix derivative in treatment of recession defects

Author, year	Defects	Subjects	Intervention	Duration	Outcomes
Abbas et al. (2003)	Miller class I recession of at least 4 mm in depth	6 patients with 6 sites	CAF + EMD	12 months	3.5 mm of root coverage (i.e., 73% root coverage, range 60–100%), 4-mm CAL gain (range 3–5 mm).
Berlucchi et al. (2002)	Miller class I or II recession of at least 2 mm depth	14 patients with 26 sites	1. CAF + EMD 2. CAF + CTG + EMD	6 months	For the CAF + EMD group, the root coverage at 6 months was 93.97%, with an attachment gain of 3.2 mm; for the CAF + CTG + EMD group, the root coverage was 93.59%, with an attachment gain of 3.4 mm. Keratinized gingiva was increased for both groups, but more for the CAF + CTG + EMD group (1.38 mm vs. 0.69 mm; $P < 0.05$).
Berlucchi et al. (2005)	Miller class I or II recession of at least 2 mm depth	30 nonsmoking patients (mean age 32.8 ± 6.2 year) with 30 sites	CAF + EMD	12 months	At 12 months, 91.7% of root coverage was obtained with a mean attachment gain of 3.23 mm. Better results in terms of percentage of root coverage were obtained when the baseline REC was <4 mm compared to defects ≥ 4 mm (96.5% vs. 83.5%). Flap thickness was positively correlated to the percentage of root coverage.

(continued)

Table 3.7 (continued)

Author, year	Defects	Subjects	Intervention	Duration	Outcomes
Castrellanos et al. (2006)	Miller class I or II gingival recessions >2 mm	22 patients with 22 sites	1. CAF+EMD 2. CAF	12 months	Vertical recessions were reduced from 2.68 ± 1.63 to 0.36 ± 0.60 mm in the test group and from 2.31 ± 1.52 to 0.90 ± 0.95 mm in the control group. Horizontal recessions decreased from 4.27 ± 2.06 to 0.77 ± 0.87 mm in the test group and from 3.68 ± 1.91 to 1.72 ± 1.31 mm in the control group. Changes in keratinized gingiva went from 3.81 ± 1.95 to 4.63 ± 2.15 mm in the test group and from 3.31 ± 1.81 to 3.27 ± 1.80 mm in the control group. The average percentage of root coverage for test and control groups was 88.6% and 62.2%, respectively. When both treatments were compared at 12 months, there was a significant difference in vertical tooth coverage and gain in keratinized gingiva in favor of the experimental group ($P < 0.05$).
Del Pizzo et al. (2005)	Miller class I or II gingival recessions ≥ 3 mm	15 patients with 2 paired defects	1. CAF+EMD 2. CAF	24 months	In the test group, gingival recession decreased from 4.07 ± 0.59 mm at baseline to 0.47 ± 0.74 mm at 24 months, corresponding to a mean root coverage (MRC) of 90.67%, whereas in the control group recession shrank from 4.13 ± 0.74 mm at baseline to 0.60 ± 0.83 mm at 24 months (MRC = 86.67%). Complete root coverage was achieved at 24 months in 73.33% and 60% of the two groups.

Hägewald et al. (2002)	Miller class I or II recession-type defects ≥ 3 mm width at least 1 mm of keratinized tissue	36 patients with 2 paired defects	1. CAF+EMD 2. CAF + placebo	12 months	Mean recession change after 12 months postoperative was 2.8 mm (SD 0.8) for the Emdogain A group and 2.9 mm (SD 0.9) for the control, representing 80% and 79%, respectively, of root coverage. The CAL gain was 3.4 mm (SD 1.1) for the EMD group and 3.1 mm (SD 1.3) for the placebo group.
McGuire and Nunn (2003)	Miller class II recession-type defects ≥ 4 mm	17 patients with 2 paired defects	1. CAF+EMD 2. CAF+CFG	12 months	The CAF + EMD was superior to the CAF + CFG with regard to early healing and patient-reported discomfort, whereas the CAF + CFG demonstrated greater amount of keratinized tissue during the 12-month evaluation period. However, both the test and control showed a significant increase in the amount of keratinized tissue at 9 and 12 months compared to baseline. No significant difference in the amount of root coverage was found between the test and control groups ($P=0.82$). The average percentages of root coverage for control and test groups were 93.8% and 95.1%, respectively. One hundred percent root coverage was obtained 89.5% of the time with CAF + EMD and 79% of the time with CAF + CFG.
Modica et al. (2000)	Miller class I or II	12 patients with 2 paired defects	1. CAF+EMD 2. CAF	12 months	The mean root coverage was 3.36 ± 1.55 mm, corresponding to a value of 91.2% for the test group, and 2.71 ± 1.20 mm equal to 80.9% for the control group ($P>0.05$). The mean CAL gain was 3.57 ± 1.55 mm for the test group and 2.79 ± 1.19 mm for the control group. No changes of PD and KT were found.

(continued)

Table 3.7 (continued)

Author, year	Defects	Subjects	Intervention	Duration	Outcomes
Nemcovsky et al. (2004)	Miller class I or II buccal recession-type defects in the anterior or premolar teeth	70 patients with 2 paired defects	1. CAF+EMD 2. CFG	12 months	At 6 months, percent of root coverage was $77.4 \pm 11.92\%$ in EMD and $84.1 \pm 11.97\%$ in CTG ($P=0.024$). At 12 months, percent of root coverage in EMD was $71.7 \pm 16.14\%$ and $87.0 \pm 12.22\%$ in CTG ($P<0.001$). Differences between the 6- and 12-month vertical recession defect and percent of root coverage recordings within each group were also statistically significant.
Pilloni et al. (2006)	Miller class I or II buccal recession	30 patients with 30 sites	1. CAF+EMD 2. CAF	18 months	The CAF+EMD group presented with significantly greater root coverage than the CAF group (2.66 ± 0.61 mm vs. 1.73 ± 0.70 mm, respectively), more CAL gain than the control group (2.80 ± 0.76 mm vs. 2.06 ± 0.70 mm, respectively) and a greater gain in the apico-coronal dimension of the keratinized tissue than the control group (0.13 ± 0.06 mm vs. -0.06 ± 0.01 mm, respectively).

Spahr et al. (2005)	Miller class I or II buccal recession	30 patients with 2 paired defects	1. CAF+EMD 2. CAF+placebo	24 months	The mean gingival recession decreased from 3.6 to 0.8 mm for the EMD-treated sites and from 3.8 to 1.4 mm for the control sites ($P > 0.05$). Similarly, all other clinical parameters did not differ significantly in the between-group comparison except for the recession width ($P = 0.027$) and probing depth ($P = 0.046$) exhibiting higher reductions in the EMD group. Complete root coverage could be maintained over 2 years in 53% of the EMD versus merely 23% in the control group. A total of 47% of the treated recessions in the control group deteriorated again in the second year after therapy compared to 22% in the EMD group. It was concluded that EMD seems to provide better long-term results.
Moses et al. (2006)	Miller class I or II buccal recession-type defects in the anterior teeth or premolars	65 patients with 65 sites	1. CAF+EMD 2. CFG	24 months	At the 12-month recording, PRC was $73.2 \pm 15.58\%$ in the EMD group and $86.8 \pm 12.48\%$ in the CTG group ($P < 0.001$). At the final evaluation, PRC increased to $76.9 \pm 16.77\%$ in the EMD group and decreased to $84.3 \pm 13.32\%$ in the CTG group ($P = 0.053$). In the EMD group, HKT increased from 1.07 ± 0.66 mm at baseline to 1.75 ± 0.59 mm at the 12-month follow-up and 2.25 ± 0.52 mm at 24 months. At the CTG sites, HKT increased from 1.65 ± 0.92 mm at baseline to 4.24 ± 0.89 mm at the 12-month recording and slightly decreased to 4.05 ± 0.94 mm at 24 months. (EMD: $P < 0.001$; CTG: $P = 0.017$).

(continued)

Table 3.7 (continued)

Author, year	Defects	Subjects	Intervention	Duration	Outcomes
Cueva et al. (2004)	Miller class I, II or III recession type defects ≥2 mm width	17 patients with 2 paired defects	1. CAF+EMD 2. CAF	6 months	There was a mean increase in keratinized tissue of 0.60 mm for test sites and a mean decrease of 0.05 mm for control sites. Test sites demonstrated significantly better root coverage ($P<0.001$), 89.7% and 92.9% root coverage after 3 and 6 months, respectively, while control sites had 56.6% and 66.8% root coverage after 3 and 6 months, respectively. There was significantly more root coverage among test sites compared to control sites, regardless of arch or Miller classification.

CAF coronally advanced flap, CAL clinical attachment level, CTG connective tissue graft, EMD enamel matrix derivative, HTK height of keratinized tissue, KT keratinized tissue, PD probing depth, PRC percentage of root coverage, SD standard deviation

Table 3.8 Systematic reviews and meta-analyses on the efficacy of enamel matrix derivative in the treatment of gingival recession

Authors	Studies included	Conclusions
Cheng et al. (2007)	7 studies for the CAF+EMD group, 4 studies for the CAF+RC group, 7 studies for the CAF group	The results of clinical attachment level, gingival recession depth and root coverage percentage in the coronally positioned flap+EMD group were statistically significantly better than the changes in the coronally positioned flap and coronally positioned flap+chemical root surface conditioning group at 6 and 12 months ($P<0.001$). There was no significant difference at the 6-month comparison among clinical attachment level, keratinized tissue, probing pocket depth and gingival recession depth, except in the root coverage percentage for coronally positioned flap and coronally positioned flap+chemical root surface conditioning groups.
Cairo et al. (2008)	5 studies for the CAF versus CAF+EMD; 1 study for CAF+CTG versus CAF+EMD	EMD in conjunction with CAF procedure enhances the probability to obtain complete root coverage and to improve recession reduction in Miller class I and II single gingival recession.
Chambrone et al. (2009)	2 studies for CAF versus CAF+EMD	There were no statistically significant differences between the EMP+CAF and the CAF in gingival recession and clinical attachment changes. However, there was a significant greater gain in the width of keratinized tissue for EMP+CAF when compared to CAF alone of 0.40 mm (95% CI: 0.09–0.71).

CAF coronally positioned flap, CTG connective tissue graft, RC root surface conditioning

60.88±5.12% root coverage percentage at 6 months and 79.00±0.00% root coverage percentage at 12 months. In the coronally positioned flap+EMD group, the results were 84.33±7.72% root coverage percentage at 6 months and 84.42±8.75% root coverage percentage at 12 months. The root coverage percentage of the coronally positioned flap+EMD group was statistically significantly better ($P<0.001$) than those of the coronally positioned flap and coronally positioned flap+chemical root surface conditioning groups at 6 and 12 months. At 6 months, the root coverage percentage of the coronally positioned flap group was statistically significantly better ($P<0.001$) than that of the coronally positioned flap+chemical root surface conditioning group. At 12 months, the root coverage percentage of the coronally positioned flap+chemical root surface conditioning group was statistically significantly better ($P<0.001$) than that of the coronally positioned flap group. This suggests that root coverage procedures in the coronally positioned flap alone and coronally positioned flap+chemical root surface conditioning procedures

were unpredictable. They became more predictable when the coronally positioned flap procedure was improved by the modification of adding EMD (Cheng et al. 2007) (Figs. 3.12 and 3.13).

Cairo et al. (2008) systematically reviewed the literature (25 included studies, 27 articles) on coronally advanced flap (CAF) alone or in combination with tissue grafts, barrier membranes, enamel matrix derivative (EMD) or other material for treating gingival recession. Regarding complete root coverage, meta-analysis (five RCT included Modica et al. 2000; Del Pizzo et al. 2005; Spahr et al. 2005; Castellanos et al. 2006; Pilloni et al. 2006) showed better results for CAF+EMD ($P=0.003$; OR=3.89; 95% CI from 1.59 to 9.50) (Fig. 3.14). CAF+EMD provided better results also for recession reduction ($P=0.002$; mean difference=0.58 mm; 95% CI from 0.21 to 0.95; Fig. 3.15), CAL gain ($P=0.0001$; mean difference=0.53 mm; 95% CI from 0.26 to 0.80; Fig. 3.16) and keratinized tissue gain ($P=0.0007$; mean difference=0.42 mm; 95% CI from 0.18 to 0.66) than CAF alone. Only one study compared CAF+connective tissue graft (CTG)

Fig. 3.12 Control site on the lower right canine treated with coronally advanced flap in a smoker patient. (a) preoperative gingival recession. (b) almost complete recession coverage 12 months after surgery (Hägewald et al. 2002. Reprinted with permission from John Wiley & Sons)

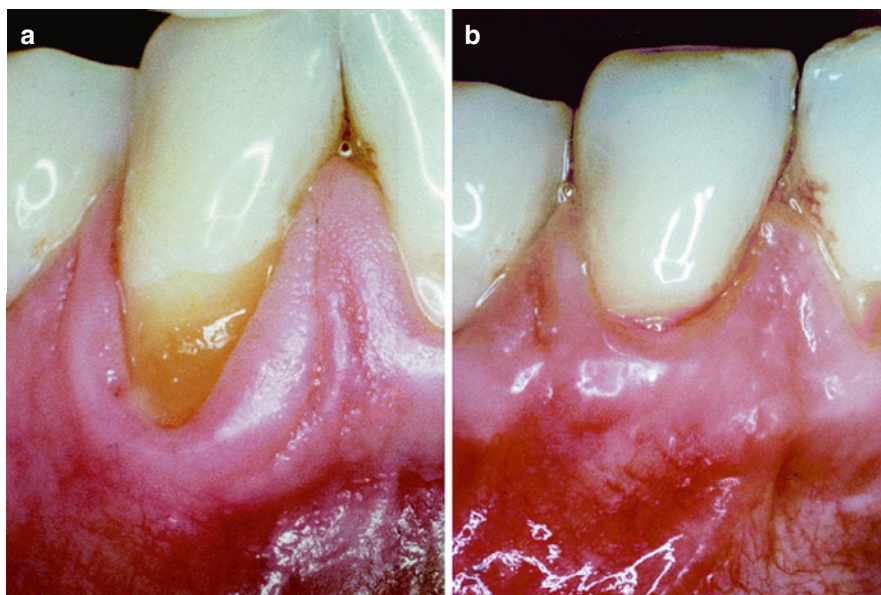
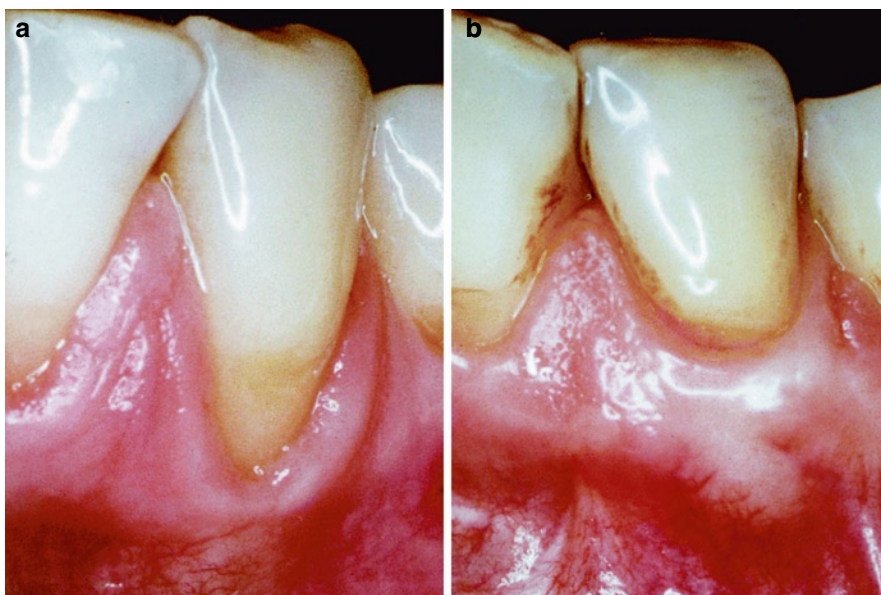


Fig. 3.13 Contralateral test site in the same patient treated with coronally advanced flap and Emdogain® application. (a) preoperative gingival recession. (b) almost complete recession coverage 12 months after surgery. Note the substantial gain in keratinized tissue (Hägewald et al. 2002. Reprinted with permission from John Wiley & Sons)



versus CAF + EMD (McGuire and Nunn 2003), reporting no significant difference ($P=0.31$; OR = 2.31; 95% CI from 0.45 to 11.74) for complete root coverage. Comparisons in terms of recession reduction and CAL gain were not possible due to data presentation in the original articles even though no statistically significant difference was reported by the authors. Also, comparison in terms of keratinized tissue gain between

CAF + EMD versus CAF + CTG was not possible due to data presentation in original articles; however, McGuire and Nunn (2003) reported higher keratinized tissue gain for CAF + CTG than EMD + CAF ($P < 0.001$) 1 year following therapy.

A recent Cochrane Systematic Review (Chambrone et al. 2009, 2010) evaluated the effectiveness of different root-coverage procedures in the treatment of

Review: Recession and CAF
Comparison: 03 CAF+EMD vs CAF
Outcome: 01 CRC

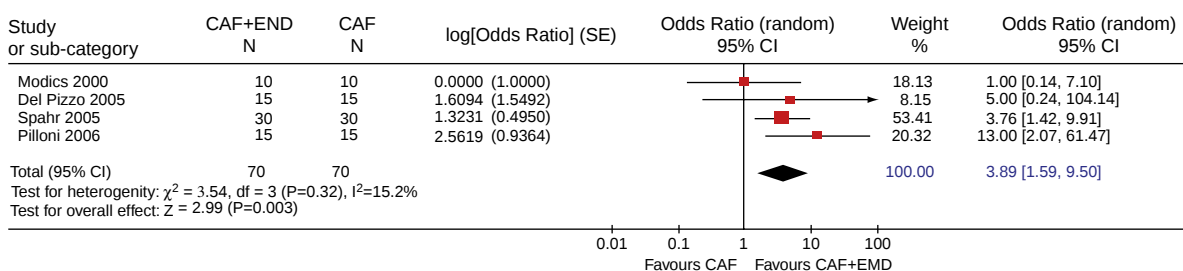


Fig. 3.14 Comparison of CAF+EMD versus CAF for CRC. CAF coronally advanced flap, CRC complete root coverage, EMD enamel matrix derivative (Cairo et al. 2008. Reprinted with permission from John Wiley & Sons)

Review: Recession and CAF
Comparison: 03 CAF+EMD vs CAF
Outcome: 02 RecRed

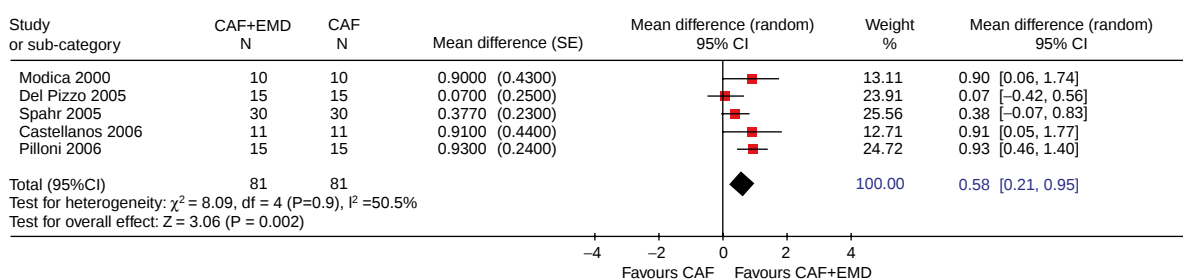


Fig. 3.15 Comparison of CAF+EMD versus CAF for change in gingival recession (RecRed). CAF coronally advanced flap, EMD enamel matrix derivative (Cairo et al. 2008. Reprinted with permission from John Wiley & Sons)

Review: Recession and CAF
Comparison: 03 CAF+EMD vs CAF
Outcome: 05 CAL Gain

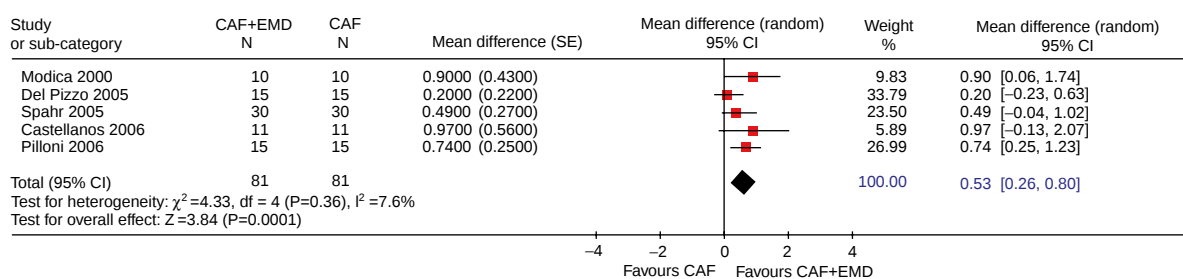


Fig. 3.16 Comparison of CAF+EMD versus CAF for CAL gain. CAF coronally advanced flap, EMD enamel matrix derivative (Cairo et al. 2008. Reprinted with permission from John Wiley & Sons)

recession-type defects. Only clinical randomized controlled trials (RCTs) with a duration of ≥ 6 months that evaluated recession areas (Miller class I or II ≥ 3 mm) that were treated by means of periodontal plastic surgery procedures were included. Twenty-four RCTs provided data.

Regarding gingival recession (GR) and clinical attachment level (CAL) changes, there were no statistically significant differences between the EMP + CAF and the CAF in gingival recession and clinical attachment changes (two trials; Del Pizzo et al. 2005; Spahr et al. 2005):

- GR change: $P=0.19$, mean difference = 0.25 mm; (95% CI: -0.13 to 0.64, $\chi^2=1.27$, $df=1$, $P=0.26$, $I^2=21.0\%$);
- CAL changes: $P=0.22$, mean difference = 0.27 mm (95% CI: -0.16 to 0.69, $\chi^2=0.36$, $df=1$, $P=0.55$, $I^2=0\%$).

However, there was a significant greater gain in the width of keratinized tissue for EMP + CAF when compared to CAF alone of 0.40 mm (95% CI: 0.09–0.71, $\chi^2=0.52$, $df=1$, $P=0.47$, $I^2=0\%$).

Sculean et al. (2007c) noted that most controlled clinical studies evaluating the treatment of gingival recessions with coronally repositioned flaps and EMD therapy reported stable clinical results after a longer time period, up to 2 years, and an increase in the width of keratinized tissue, thus indicating that EMD may have an effect upon the proliferation and keratinization of gingival fibroblasts (Spahr et al. 2005; Del Pizzo et al. 2005; Moses et al. 2006).

3.9 Clinical Studies Evaluating the Effect of EMD on Early Wound Healing

EMPs cause a stimulation of total protein synthesis and synthesis of specific extracellular matrix molecules (glycoproteins and proteoglycans). Overall, EMPs downregulate the expression of genes involved in early inflammatory events of wound healing and upregulate the expression of genes encoding growth and repair-promoting molecules. The type of molecule affected by EMP treatment appears to depend on the cell type and differentiation/maturation state. Among the upregulated molecules are TGF- β 1, BMP-2, BMP-7, PDGF-AB, VEGF, CTGF, FGF-2, IGF-1, TNF- α , IL-6, IL-8, PGE2, OPN, collagen types II and X, MMP-2 and ALP. In particular, BSP, OC and type I collagen showed inconsistent results (Bosshardt 2008).

Several studies have attempted to evaluate the effect of EMD treatment on early wound healing (Wennström and Lindhe 2002; Hagenaa et al. 2004; Okuda et al. 2001). Based on the available data, at the current time, it appears that no definitive conclusions can be drawn regarding the extent to which additional application of EMD may further enhance early wound healing following conventional periodontal therapy (Sculean et al. 2007d).

3.10 Clinical Studies Evaluating the Effect of EMD on Periodontal Healing of Replanted Teeth

Tooth avulsion is a complex injury affecting pulp, periodontal ligament (PDL), cementum layer and alveolar bone and is usually followed by pulpal and periodontal complications, which might compromise tooth survival. Complicating sequelae of pulp necrosis, damaged PDL and cementum layer may result in external inflammatory resorption or replacement resorption, which may ultimately result in tooth loss (Wiegand and Attin 2008).

Enamel matrix derivative (EMD) has attracted interest to improve periodontal healing of avulsed and replanted teeth, as it influences the migration, attachment, proliferative capacity and biosynthetic activity of periodontal ligament cells (Wiegand and Attin 2008; Bosshardt 2008).

The time elapsed between a trauma and tooth replantation usually ranges from 1 to 4 h. The chances of root surface damage are higher when tooth replantation is not performed immediately or if the avulsed tooth is not stored in an adequate medium. This invariably leads to necrosis of pulp tissue, periodontal ligament cells and cementum, thus increasing the possibility of root resorption, which is the main cause of loss of replanted teeth (Panzarini et al. 2008).

The International Association of Dental Traumatology (IADT) has developed guidelines for the treatment of traumatic dental injuries. For avulsed permanent teeth with closed apex and with extra-oral dry time >60 min it was recommended that, after mechanical removal of periodontal ligament remnants, the teeth should be immersed in an acidulated sodium phosphate fluoride solution at 2.4%, pH 5.5, for a minimum of 5 min or, if available, fill the socket with Emdogain (Flores et al. 2001).

Several human case series or case reports (Barrett et al. 2005; Ninomiya et al. 2002; Al-Hezaimi et al. 2009; Hamamoto et al. 2005; Guzmán-Martínez et al. 2009; Çağlar et al. 2005; Filippi et al. 2001, 2006) have revealed the beneficial or limited (Chappuis and von Arx 2005) effect of Emdogain in the treatment of avulsed teeth. However, Schjøtt and Andreasen (2005) showed that Emdogain was not able to prevent or cure ankylosis, while Molina and Brentegani (2005), Araújo

et al. (2003) Lam, and Sae-Lim (2004) demonstrated that Emdogain was unable to stimulate tissue repair in reimplanted teeth or to reduce replacement resorption.

3.11 Advantages of the Use of Emdogain Gel

Even if many studies have demonstrated that the treatment of intrabony defects with GTR with non-resorbable and resorbable membranes has a high percent of success, this procedure has several disadvantages. Membrane application is a time-consuming and technique-sensitive procedure and must be performed very carefully. Moreover, trimming, suturing and tight adaptation of non-resorbable and resorbable membranes may be difficult, especially in the posterior areas of the mouth. If a non-resorbable membrane is used, a second surgical procedure is required to remove the membrane. Such a procedure may cause gingival recession as an effect of marginal necrosis of the flap, creating the need for an additional surgical procedure aimed at harvesting a connective tissue or free gingival graft to cover the newly formed tissue. Furthermore, GTR requires a very intensive follow-up, especially when suppuration at surgical site or membrane exposure occurs (Silvestri et al. 2000).

When using the GTR technique, healing is obtained by means of (1) clot protection, (2) use of barrier that allows the deep periodontium cells to reproduce without interference from the superficial periodontium and (3) empty space between membrane and underlying tissues that allows the new tissue to grow within the defect. These rules have little significance when using Emdogain because this product theoretically reproduces a morphogenetic process that occurs in nature at the time of development of the dental organ. This fact affects both the type and timing of healing (Parodi et al. 2000).

The use of EMD gel has a number of advantages in clinical practice in comparison with other regeneration technologies. The application of EMD gel during periodontal surgery is easier and faster than most other products available, while postoperative healing such as swelling and pain are minimal. Another advantage is the possibility of treating multiple adjacent defects with one surgical procedure and one vial of EMD gel

(Parashis and Tsiklakis 2000). Furthermore, the problems associated with membrane exposure are absent with EMD (Sculean et al. 2002a). The use of EMD can be helpful in favoring resolution of deep infrabony defects, especially in esthetically sensitive sites, due to the limited increase of gingival recession after surgery (Zucchelli et al. 2002).

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Chemical root conditioning is widely used to improve the outcome of regenerative periodontal therapies by favoring the attachment of the regenerated periodontal structures (Vanheusden et al. 1999).

Periodontitis affected root surfaces are known to be hypermineralized and may be contaminated with periodontal pathogens and endotoxins contained within dental calculus and root cementum (Aleo et al. 1974; Adriaens et al. 1988; Mayfield et al. 1998). The purpose of scaling and root planing to remove cytotoxic substances contained in dental calculus and contaminated cementum on root surfaces is not a realistic objective for periodontal therapy, because of two main reasons: first, bacterial toxins are not completely eliminated from the root surface (Kepic et al. 1990), and second because a smear layer is left by mechanical instrumentation, which act as a physical barrier, inhibiting cell re-attachment and serving as a reservoir for bacterial growth (Polson et al. 1984; Blomlöf and Lindskog 1995). Therefore, it has been proposed to chemically condition the roots in order to improve their biological compatibility (Vanheusden et al. 1999).

Chemical root surface conditioning has been introduced, using a variety of agents, in order to detoxify, decontaminate and demineralize the root surface, thereby removing the smear layer and exposing the collagenous matrix of dentin and cementum (Vanheusden et al. 1999; Blomlöf 1996).

An underestimated issue may be the mechanical strength between the treated root surface and new cementum. Tissue separation between new cementum and the treated root surface is a very common finding in experimental periodontal regeneration studies (reviewed by Schroeder 1992). Thus, appropriate root surface conditioning may not only provide a biocompatible surface for cell attachment, cell spreading and

matrix deposition, but might also improve mechanical interfacial bonding, and therefore could be an issue of clinical relevance (Bosshardt and Sculean 2009).

Various acids have been used for chemical root surface conditioning, including citric and phosphoric acids, ethylenediaminetetraacetic acid (EDTA) and tetracycline hydrochloride (as reviewed by Lowenguth and Blieden 1993; Mariotti 2003; Wang et al. 2005). These procedures in an animal model are believed to be able to induce cementogenesis and enhance attachment either by connective tissue in-growth and/or demineralization. However in human studies, no clinical advantages were observed. The clinical relevance of root conditioning with an acid agent in routine periodontal surgery is still questionable (Cheng et al. 2007).

4.1 Citric Acid

Root surface demineralization with citric acid has been suggested to be used as a part of regenerative procedures because of the ability of citric acid to modify the root surface (Register and Burdick 1975, 1976). Topical application of citric acid causes a superficial demineralization of the root surfaces, eliminates bacterial endotoxins, is partly bactericidal (Daly 1982) and partially exposes collagen matrix of the radicular dentin to a depth of 3–10 mm (Garrett et al. 1978). This latter effect has been shown to increase collagen splicing, improve fibrin linkage and consequently inhibit epithelial downgrowth; to stimulate fibroblast attachment and migration; and to facilitate new cementum formation (Vanheusden et al. 1999). The in vitro test of the consequences of conditioning human dentine by citric acid or minocycline suggest

that these two substances could promote the attachment, the proliferation and the biosynthetic activity of human periodontal ligament, prerequisites to periodontal regeneration (Rompen et al. 1999).

Recently, Ruggeri et al. (2007) evaluated, with an immunohistochemical approach, the labeling pattern of collagen fibrils and chondroitin sulfate subsequently exposed by dentin root surface chemical treatment after manual (scaling and root planing) or ultrasonical instrumentation (Figs. 4.1 and 4.2). The findings of this study confirmed that citric acid removes the smear layer after instrumentation and unveils numerous collagen fibrils and associated proteoglycans as confirmed by intense and positive immunohistochemical labeling. Similarly, EDTA demineralization removes the smear layer but leaves the inorganic matrix onto the dentin matrix determining a reduced labeling pattern when compared with citric acid etched specimens (Ruggeri et al. 2007).

When the adhesion and maturation of blood components on chemically conditioned root surfaces were compared, citric acid was indicated to stabilize clots on the root surface, which act as a scaffold for connective tissue cell development. EDTA showed a moderate fibrin network formation. In contrast, a scarce fibrin network and few cells were present in the tetracycline samples, and an absence of blood elements was found on sodium citrate specimens (Leite et al. 2010).

Even if experimental studies in dogs using citric acid demineralization of root surfaces showed new connective tissue attachment (Crigger et al. 1978; Nilvéus et al. 1980; Nilvéus and Egelberg 1980; Klinge et al. 1981), the controlled clinical trials in humans did not reveal any adjunctive clinically observed effects when citric acid treatment was used

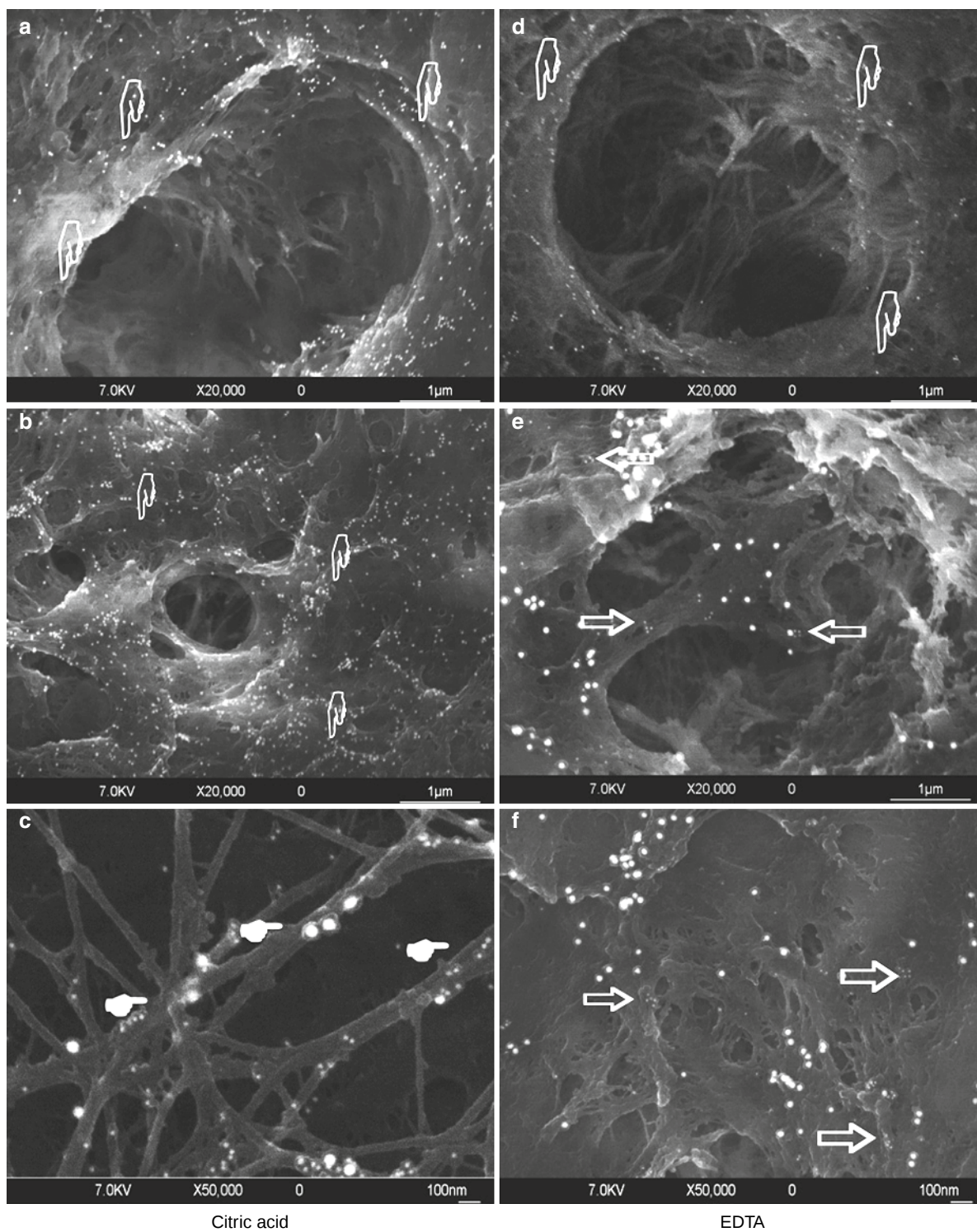
in conjunction with surgical procedures, either without or in combination with osseous grafts or GTR techniques (Stahl et al. 1983; Renvert et al. 1985; Moore et al. 1987; Handelsman et al. 1991; Kersten et al. 1992; Fuentes et al. 1993; Lowenguth and Blieden 1993; Klinge 1996). The evaluation of clinical and microbiological effects of citric acid irrigation as a supplement to scaling and root planing with culture samples revealed that the sites treated with a combination of scaling and irrigation with citric acid demonstrate a similar healing pattern as sites treated with scaling and root planing alone (Renvert et al. 1997). However, histologic evaluation in a human clinical trial provided evidence of fibrous attachment following citric acid demineralization. Connective tissue was apposed directly to old or newly formed cementum, but never directly to dentin. Fibrous attachment was usually functionally oriented, i.e., perpendicular to the root surface (Albair et al. 1982).

The review performed by Mariotti (2003) included 14 quasi experimental clinical studies and 4 case reports. The length of citric acid application to the root surface ranged from 20 seconds to 5 minutes and study duration was between two and five years. The meta-analysis of seven studies (Blomlöf et al. 2000b; Caffesse et al. 1987; Handelsman et al. 1991; Kersten et al. 1992; Moore et al. 1987; Parodi and Esper 1984; Smith et al. 1986) revealed that the application of citric acid to single or multirrooted teeth with soft or hard tissue grafts, flaps or membranes was found as effective in improving attachment levels as non acid-treated controls (Mariotti 2003).

Attempts to combine root surface demineralization and fibronectin to induce a more significant regenerative

Fig. 4.1 FEISEM images of root surfaces treated with scaling and root planing and treated with citric acid (Group A1) or EDTA (Group A2). The images were obtained by mixing the back-scattered signal showing the gold nanoparticles as spheric white spots of 15 and 30 nm in diameter with the secondary electron signal showing surface morphology. (a) Dentin surface after etching with citric acid (Group A1) that effectively removes the superficial mineral phase revealing dentin tubules and the peritubular fibrillar network. The smear layer is fully removed. Labeling of collagen fibrils is detectable as 30 nm white spots scattered along the fibrils (*pointers*). (b) Intertubular dentin surface after etching with citric acid (Group A1). Collagen fibrils are clearly labeled by the monoclonal antibodies (*pointers*) revealing a well-preserved three-dimensional arrangement. (c) High magnification view of intertubular dentin surface of specimens of Group

A1 showing minor branching fibrils of approximately 40 nm in diameter. Positive labeling of 15 nm white spots is identifiable revealing proteoglycans associated to major collagen fibrils (*arrows*). (d) FEISEM image of EDTA conditioned specimen (Group A2) revealing patent dentinal tubules with major exposed fibrils. No residual smear layer is detectable. Peritubular dentin appears as a thick collar around the tubular opening (*pointers*). (e) Intertubular dentin surface (Group A2) reveals positive labeling for both collagen fibrils (30 nm white spots) and associated proteoglycans (15 nm spots). (f) Superficial porosity of EDTA treated intertubular dentin surface (Group A2) is reduced compared with citric acid etched specimens (Group A1; c) as well as gold labeling. Proteoglycans are sometimes clustered in globules as identified by *arrows* (Ruggeri et al. 2007. Reprinted with permission from Elsevier)



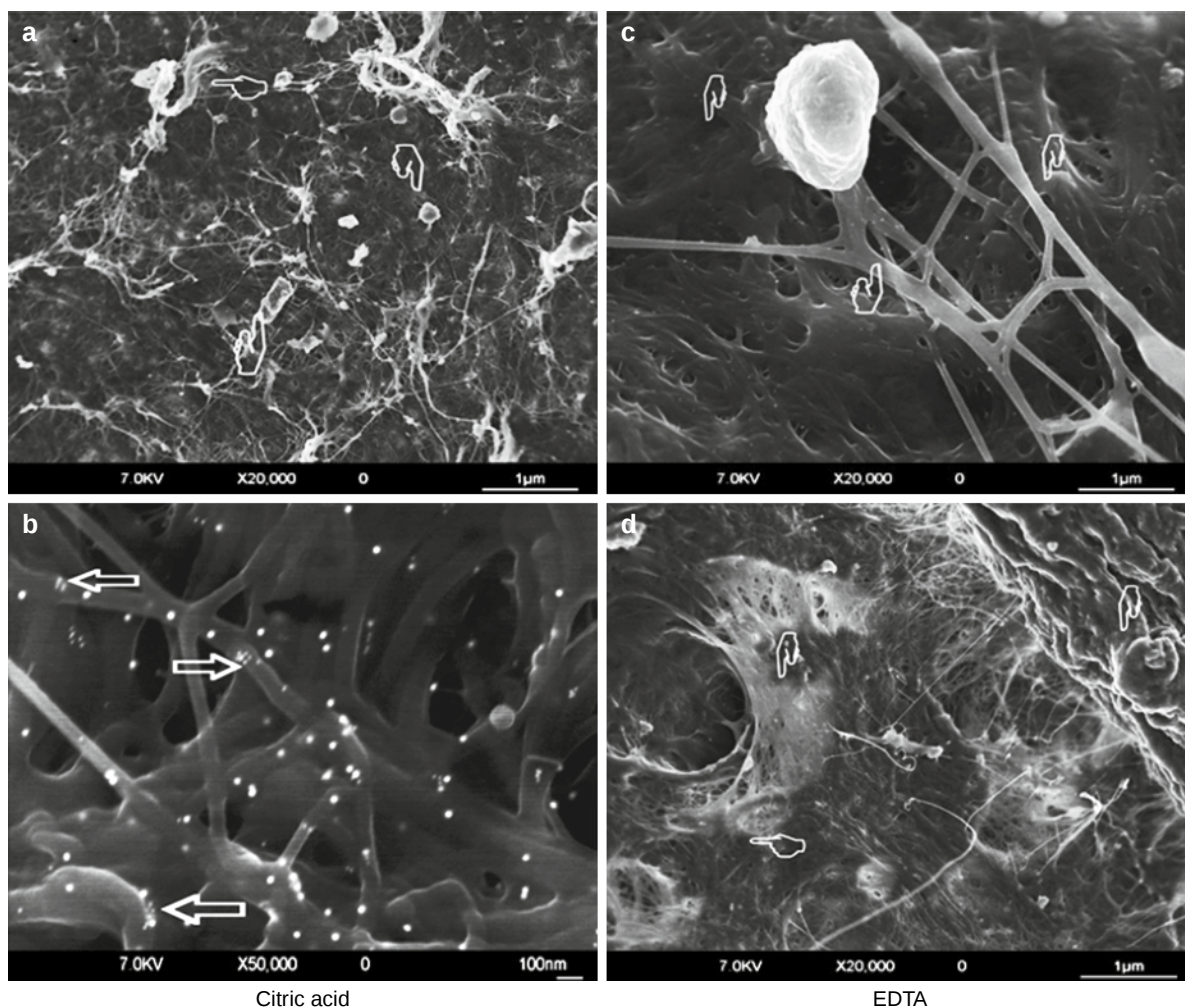


Fig. 4.2 FEISEM images of dentin surface after ultrasonic instrumentation, and either citric acid (Group B1) or EDTA (Group B2) exposure and immunocytochemical labeling of collagen and proteoglycans. (a) Low magnification image reveals residual organic debris scattered on the surface (*pointers*) after citric acid etching (Group B1). (b) Citric acid exposure produces porous intertubular zones revealing the collagen fibrillar network with intense labeling for both collagen and proteoglycans

(*arrows*). (c) After EDTA conditioning (Group B2), organic debris are sometimes detectable on the surface exposed to ultrasonic instrumentation (*pointers*). (d) The association of ultrasonic instrumentation and EDTA surface treatment (Group B2) was able to open tubule orifice, whereas the peritubular dentin always reveal a thick and compact aspect surrounding the orifice. Labeling is scarce and scattered (Ruggeri et al. 2007. Reprinted with permission from Elsevier)

response have shown promise in promoting reattachment after periodontal therapy in animal (Caffesse et al. 1985; Smith et al. 1987a, b) and human clinical studies (Caffesse et al. 1988).

Regarding citric acid adverse effects, studies in monkeys indicated that acids used at low pH (citric and phosphoric) have a necrotizing effect on both mucosal flaps and periodontal tissues (Blomlöf and Lindskog 1995). Root resorption has also been reported following root conditioning with citric acid (Klinge et al. 1985).

4.2 Tetracycline HCl

The potential of Tetracycline Hydrochloride (TTC-HCl), and more specifically as a demineralizing agent in root conditioning, was analyzed by several studies. TTC-HCl showed higher ability to affect both dentin smear layer removal and tubule exposure compared to minocycline and doxycycline (Madison and Hokett 1997; Shetty et al. 2008). The migration of periodontal ligament cells is enhanced when dentin is preconditioned

with TTC-HCl. Scanning electron microscopic studies have shown that after burnishing, the results are comparable to citric acid: dentinal tubules and the dense network of collagen fibers that make up the dentin structure are exposed by the removal of the smear layer (Lafferty et al. 1993; Babay and Mokeem 2005). However, a relatively unhealthy appearance of periodontal fibroblasts on root surfaces treated with TTC-HCl was reported (Chandra et al. 2006). Furthermore, dentin surfaces treated with TTC-HCl may bind fibronectin more easily than those treated with citric acid (Terranova et al. 1986) and promotes fibroblast adhesion and growth (Rompen et al. 1999).

An in vitro study assessed smear layer removal and collagen fiber exposure after TTC-HCl application on root surfaces using Scanning Electron Microscopy (SEM). Root cementum was removed with diamond burs followed by scaling and root planing. Four hundred fifty samples were divided into ten groups: a “control” (saline application) and nine different TTC-HCl concentrations were applied at doses of 10, 25, 50, 75, 100, 125, 150, 200 and 250 mg/mL. The concentrations of 50 and 75 mg/mL applied by burnishing were the most effective, suggesting that these parameters may be applied in periodontal procedures involving TTC-HCl root conditioning to optimize results (Ishi et al. 2008; Isik et al. 2000). Time-dependent changes were observed in dentin surfaces, the intertubular “matted” collagen matrix being evident only after a four-minute application (Trombelli et al. 1994; Hanes et al. 1991; Babay 2001).

Contradictory results were reported in animal models. In dogs, horizontal periodontal defects were surgically induced around the mandibular premolars followed by a six-week period without plaque control. Reconstructive surgery of the defects was subsequently carried out. The root surfaces were debrided and superficially demineralized with citric acid or TTC-HCl, with or without subsequent application of fibronectin. Mucoperiosteal flaps were raised to cover most of the crowns and sutured. The animals were sacrificed 12 weeks after surgery and block sections of the teeth and surrounding tissues were processed for histology. It was reported that citric acid conditioning of the root surface frequently resulted in complete connective tissue repair of the furcation defect; similar potential to induce connective tissue repair was obtained for citric acid and TTC-HCl treatment (Wikesjö et al. 1988).

Minimal differences between citric acid and TTC-HCl treatments were reported by other authors and neither was advantageous over the application of sterile water when used in the treatment of periodontal surgical defects in dogs with or without membranes (Sammons et al. 1994; Dyer et al. 1993; Wang et al. 1993a, b; Claffey et al. 1987). When healing of experimental dehiscence defects after surface demineralization with TTC-HCl was histologically evaluated in monkeys, it was reported that root conditioning with 10% tetracycline solution did not produce any additional new attachment in comparison to the controls (Nagata et al. 2005).

The meta-analysis of three clinical studies (Darhous et al. 1995; Machtei et al. 1993; Parashis and Mitsis 1993) performed by Mariotti (2003) reported no clinical change in attachment levels, when tetracycline was used. The TTC-HCl used in these studies was most often applied in a concentration of 100 mg/mL to the root surface for 3–5 min. The study duration ranged from 3 to 12 months (Mariotti 2003).

Root resorption has been reported following root conditioning with TTC-HCl in animals (Claffey et al. 1987; Wikesjö et al. 1988, 1992) and humans (Ben-Yehouda 1997). Moreover, it was demonstrated that conditioning of diseased cementum with TTC-HCl may induce an intense inflammatory response in a mouse model (augmented levels of TNF-alpha and IFN-gamma, and reduced levels of IL-10, compared with untreated diseased cementum), suggesting that local application of tetracycline for root conditioning should be carefully reinvestigated (Hourri-Haddad et al. 2004).

4.3 EDTA

The neutral pH calcium chelator, EDTA, has a profoundly higher capacity to selectively expose collagen fibers in both healthy cementum surfaces and periodontitis-affected dentin surfaces, compared to agents operating at low pH which seemed to erode the surfaces to varying degrees (Blomlöf 1996). EDTA preserves the vitality of tissues with direct contact, and removes hydroxyapatite from the collagenous dentin matrix more selectively than low pH etching agents (Klinge 1996; Babay 2000). The effectiveness for smear layer removal of etching of the root surface with

EDTA-T gel providing the most desirable root surface to which maximum periodontal ligament cells can adhere and on which they can grow was recently reported (Carvalho Batista et al. 2005; Gamal and Mailhot 2003).

Bergenholtz and Babay (1998) suggested that the mode of application also affects the tooth surfaces. Scanning electron microscopy of root surfaces that had been ultrasonically scaled and subjected to various conditioning regimens revealed the presence of two distinct types of cracks: extensive cracks, presumed to have been caused by drying before and during sputter-coating procedures; and smaller cracks that reflected the pattern of the irregular underlying dentin. Both etching and chelating agents appear to cause demineralization of the interfacial layer between cementum and dentin, causing a “peeling-off” of cementum and an exposure of the underlying dentin. The author suggested that burnishing the scaled root surface with either saline or any of the etching or chelating agents for at least 10 seconds, followed by soaking the cementum in 8% ethylenediaminetetraacetic acid for about 40 seconds, achieved a root surface that might be regarded as optimal for regeneration of periodontal tissues.

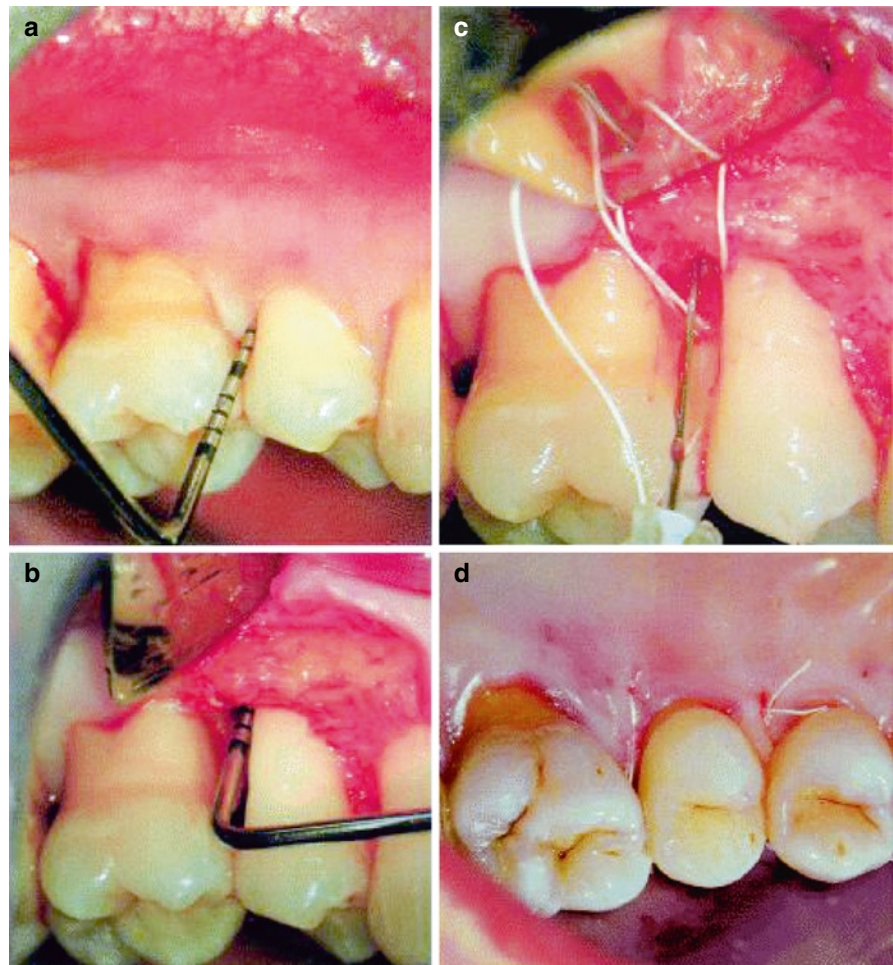
The evaluation of an animal model on periodontal healing of EDTA etching agent compared to a citric acid agent was performed by Blomlöf (1996). Maxillary molars and premolars, in total 32 teeth in four monkeys, were divided between test (EDTA or citric acid treatment) and matched control groups. Periodontal surgery on both palatal and buccal roots using the dehiscence model was performed with or without root surface etching. Healing results were evaluated histomorphometrically after eight weeks. Healing was described by expressing the length along the root surface of various healing reactions as a percentage of the total length of the experimental defect. The statistically significant differences between EDTA treated surfaces and control surfaces were approximately 10% less failure (gingival recession and periodontal pocket), 10–15% more total histological attachment (long epithelial junction, connective tissue and reparative cementum), approximately 20% less long epithelial junction and approximately 20% more connective tissue in roots etched with EDTA. In the citric acid etched roots there were approximately 10% more connective tissue and 15% less long epithelial junction compared with control. Thus etching with EDTA appeared to

improve healing, avoiding the superficial necrotizing effect on exposed periodontal tissues by citric acid. de Vasconcellos et al. (2006) investigated by means of histological and histomorphometric analysis, the effects of 24% EthyleneDiamineTetraacetic Acid (EDTA) gel in periodontal tissue when used in combination with conventional periodontal treatment. The results showed that the EDTA gel did not promote additional necrotizing effects in the cells of periodontium and connective tissue.

Despite these promising results, the meta-analysis of three studies (Blomlöf et al. 2000a, b; Mayfield et al. 1998) performed by Mariotti (2003) revealed no difference in probing depth, clinical attachment level, gingival recession or probing bone levels between a solution containing 24% EDTA treatment and control root surfaces.

In most clinical studies, root surface conditioning with EDTA was performed in conjunction with the application of Enamel Matrix protein Derivative (EMD), and therefore it cannot be excluded that the results may also be attributable to the effect of the root conditioning procedure (Fig. 4.3). Sculean et al. (2006) determined the effect of root conditioning on the healing of intrabony defects treated with EMD. Twenty-four patients, each of whom exhibited one deep intrabony defect, were randomly treated with either Open Flap Debridement (OFD) followed by root surface conditioning with EDTA and application of EMD (OFD + EDTA + EMD) or with open flap debridement and application of EMD only (OFD + EMD). The following parameters were recorded at baseline and at 1 year: Plaque Index (PI), Gingival Index (GI), Bleeding On Probing (BOP), Probing Depth (PD) and Clinical Attachment Level (CAL). No differences in any of the investigated parameters were observed at baseline between the two groups. At 1 year after therapy, the OFD + EDTA + EMD group showed a reduction in mean PD from 9.3 ± 1.3 mm to 4.0 ± 0.9 mm ($P < 0.001$), and mean CAL changed from 10.8 ± 2.2 mm to 7.1 ± 2.8 mm ($P < 0.001$). In the OFD + EMD group, mean PD was reduced from 9.3 ± 1.2 mm to 4.2 ± 0.9 mm ($P < 0.001$), and a change in mean CAL from 11.0 ± 1.7 mm to 7.3 ± 1.6 mm ($P < 0.001$). As there were no significant differences in any of the investigated parameters between the two groups, it was concluded that in intrabony defects, regenerative surgery including

Fig. 4.3 (a) Photograph of an intra-bony defect; the periodontal probe tip is inserted into the pocket. The picture was taken before surgery was started. (b) Intra-surgical probing at the defect site after flap elevation. (c) Root conditioning; untied sutures are visible. (d) Palatal view of the surgical site after the suture was closed (Francetti et al. 2004. Reprinted with permission from John Wiley & Sons)



OFD + EDTA + EMD failed to show statistically significant differences in terms of PD reduction and CAL gain, compared to treatment with OFD and application of EMD only.

Similar results were obtained by Parashis et al. (2006), who suggested that clinical and radiographic outcomes of intrabony defect enamel matrix derivative therapy do not depend on the use of EDTA gel root conditioning.

Cheng et al. (2007), in a recent systematic review, also confirmed that clinical outcomes for root coverage do not depend on the use of root conditioning. There were no statistically significant differences between coronally positioned flap-alone group and the coronally positioned flap + chemical root surface conditioning group in the amount of root coverage and increase of clinical attachment level at six months.

Limited information is available with respect to root resorption following root conditioning with EDTA (Blomlöf et al. 1996; Mayfield et al. 1998).

In summary, human trials with root surface demineralization have yet to show significant clinical improvement when compared to non-demineralized controls. Histologic evidence seems to suggest that new connective tissue attachment and limited regeneration may result from root surface demineralization. However, this histologic healing pattern does not result in significant improvement in clinical conditions beyond non-demineralized control sites. Conditioning of root surfaces appropriately is likely to be important for enhancing predictability of regenerative therapies. Research focused on identifying factors that can detoxify roots and also influence appropriate cell attachment is needed to identify appropriate root conditioning therapies (Wang et al. 2005).

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Through life, our bodies suffer insults from injuries, diseases and aging, which all might cause tissue defects or degeneration. Regrettably, the innate biological potential for repair and regeneration decreases with age, and thus tissue damage may become permanent. In perspective, regenerative or rejuvenating therapy has become one centerpiece of biomedical research and includes craniofacial indications. Early on, scientists focused on creating a suitable environment that favored the innate potential for regeneration. However, complex clinical protocols and extended treatments, in addition to inconsistent results, often brought treatment protocols out of favor. Predictable outcomes and minimally invasive protocols have become fundamental to clinicians and patients. Thus, novel regenerative concepts with improved or superior outcomes, predictability and minimally invasive protocols are being developed and considered (Huang et al. 2008).

For many years, research has attempted to use biologically active molecules to achieve periodontal regeneration. Among these molecules are extracellular matrix proteins and cell-attachment factors; mediators of cell metabolism and activity; and growth differentiation factors (GDFs) (Trombelli and Farina 2008; Bosshardt and Sculean 2009) (Fig. 5.1). Polypeptide growth factors (GFs) represent a class of biological mediators that regulate critical cellular activities, including migration, proliferation, differentiation and matrix synthesis (Table 5.1). Since the late 1980s, there has been a concerted effort to increase the knowledge of how polypeptide growth factors influence the repair and regeneration of tissues. These naturally occurring ligands have been shown to have pleiotropic effects; they support regeneration in several settings and accelerate healing processes. They exert their effects by binding to specific cell membrane receptors to initiate complex cascades that eventually reach a nuclear target

gene to generate signals for specific phenotype expression (Huang et al. 2008). Numerous growth factors, alone or in combination, have been tested for periodontal regeneration in animal experiments. Among these are insulin-like growth factors (IGFs), fibroblast growth factors (FGFs), epidermal growth factor, platelet-derived growth factors (PDGFs), vascular endothelial growth factor (VEGF), parathyroid hormone, transforming growth factor- β and bone morphogenetic proteins. In addition, the clinical effectiveness of recombinant human platelet-derived growth factor-BB, platelet-rich plasma (PRP) and peptide P-15 has also been evaluated (Nakahara, 2006; Trombelli and Farina 2008; Bosshardt and Sculean 2009).

5.1 Growth Factor Delivery for Oral and Periodontal Tissue Engineering

Providing appropriate growth factors (GFs), cells and a scaffold is necessary for most tissue-engineering approaches to reproduce the developmental sequence of overlapping events that occur during tissue formation and growth. Clinically successful therapies based on the tissue engineering concept are therefore highly dependent on the development of biomimetic material niches, the delivery of exogenous GFs and the transplantation of essential cell sources that offer renewed hope for the regeneration of lost tissues. Therefore, it is the aspiration that, in the future in periodontal regenerative medicine, tissue engineering will provide a variety of laboratory-made products (stem cells, material niches, tissue constructs or engineered tissues) for clinical therapies and transplantation (Chen et al. 2010).

To be effective as a drug, a GF has to reach its site of action without damage of its bioactivity. Then, the GF

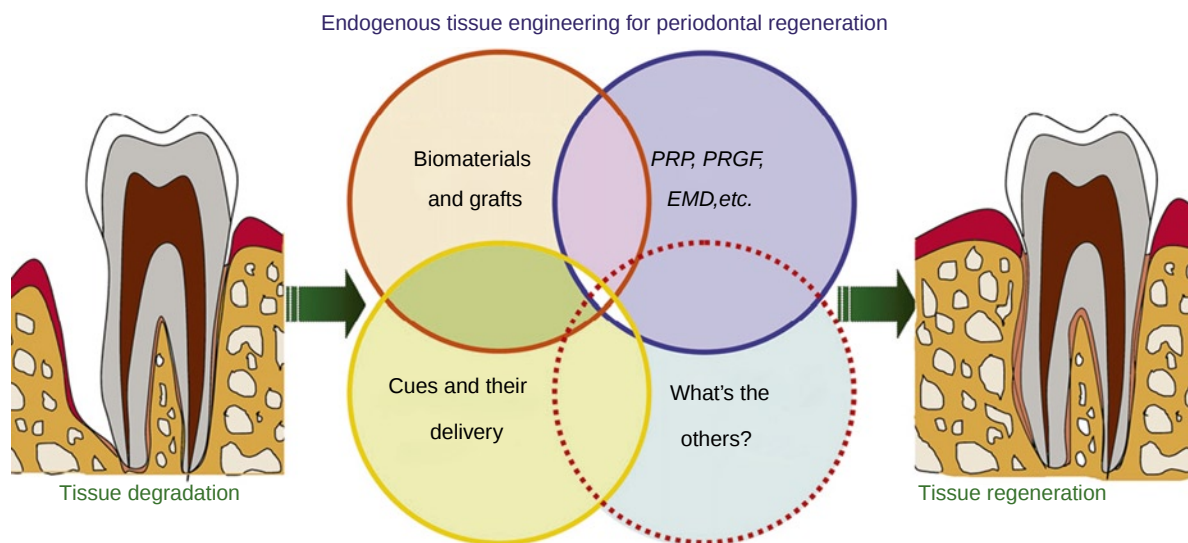


Fig. 5.1 Schematic representation of several known elements (tools) that are involved in endogenous tissue-engineering approaches to periodontal regeneration. Normally, reconstruction of lost periodontal tissue requires the combination of cells, biomaterials, and signaling cues. Other than cell transplantation, the endogenous tissue-engineering approach recruits local progenitor/stem cells from the remaining healthy periodontal tissues by providing appropriate bioactive cues via biomaterial scaffolds or delivery systems. The delivery of growth factors,

commercially available products such as enamel matrix derivative (Emdogain®, EMD) and Geistlich Bio-Oss®, or patient-derived preparations such as platelet-rich plasma (PRP) and other preparations rich in growth factors (PRGF), via minimally invasive surgical procedures to the periodontium is increasingly important in periodontal regenerative medicine, wherein cell homing, rather than cell delivery, may accelerate clinical translation (Chen et al. 2010. Reprinted with permission from Elsevier)

has to be present in the target location for a sufficiently long period of time to exert its action(s) (Kaigler et al. 2006; Chen et al. 2009, 2010 and references therein). Results from systemic administration of growth factors are often unpredictable, probably due to their short biological half-life, lack of long-term stability, tissue specificity and potential dose-dependent carcinogenicity. The carrier primarily acts as a local regulator to control doses and kinetics of released growth factor, thus increasing their potential retention time at therapeutic concentration levels. However, importance of the carrier is not limited to such roles. Recently, the role of carriers was extended to serving as a temporary substrate and three-dimensional matrix for cellular infiltration, in which cells can grow and become particular tissue types in concert with degradation of the carrier material (Lee and Shin 2007).

At present, growth factor delivery in tissue engineering and regenerative medicine basically relies upon two strategies (Chen et al. 2009):

- Direct delivery by incorporation into a vehicle
- Gene delivery

A number of proteins and polypeptides implicated in distinct stages of the bone and cartilage repair have been incorporated into the carrier accordingly via non-covalent and covalent binding (Lee and Shin 2007). Analysis of research published to date suggests that the clinical success of growth and differentiation factors for periodontal regeneration appears, to a large degree, dependent upon the characteristics of the specific carriers (Chen et al. 2009). Supporting matrices for engineering bone and soft tissue have included processed bone allografts, synthetic and natural polymers, synthetic ceramics, bovine type I collagen and calcium sulfate and have been formulated into porous scaffolds, nanofibrous membranes, microparticles and hydrogels. Bioresorbable polymers of polylactide-*co*-glycolide and polyglycolic acid have been considered as scaffolding agents for tissue engineering because of their biodegradable and tissue-compatibility properties (Kao et al. 2009). There is evidence that a combination of several materials may offer the best opportunity for beneficial clinical outcomes. Composites of different materials in the drug delivery arena are used to optimize the individual benefits of each material and improve the efficiency

Table 5.1 *In vitro* effects of growth factors on periodontal ligament cells and osteoblasts

		Platelet-derived growth factor	Fibroblast growth factor-2	Bone morphogenetic proteins	Enamel matrix derivative	Transforming growth factor-beta	Insulin-like growth factor-1, 2
Periodontal ligament cells	Cell proliferation	++	+++	++	++	-	+
	Chemotaxis	++	+++	+	++	0	++
	Collagen synthesis	+	-	+	+	+	+
	Protein synthesis	+	+	+	+	+	+
	Matrix gene expression	++	++/-	?	+	+	+
Cementoblasts	Cell proliferation	+++	?	-	++	++	++
	Chemotaxis	++	?	?	?	?	?
	Collagen synthesis	+	?	++	++	+	+
	Protein synthesis	+	?	++	++	+	+
	Matrix gene expression	+/-	?	++	++/-	+/-	+/-
Osteoblasts	Cell proliferation	++	+++	0	++	+++	++
	Chemotaxis	+++	+++	+	++	+++	+
	Collagen synthesis	0	++	0	+	++	+
	Protein synthesis	0	+	ND	+	+/-	0
	Matrix gene expression	+/-	++/-	++	++/-	++	++
	Alkaline phosphatase synthesis	0	-	++	++	+/-	0

Source: Kao et al. (2009). Reprinted with permission from John Wiley & Sons
 -, inhibition; 0, no effect; +, effect; ?, unknown effect; ND, not determined

of GF delivery and bioactivity (Kaigler et al. 2006; Chen et al. 2009, 2010 and references therein).

The major roles for the supporting matrices are listed below (Kao et al. 2009):

1. To provide physical support for the healing area so that there is no collapse of the surrounding tissue into the wound site. Examples of this would be bone allografts and synthetic ceramics such as tricalcium phosphate.
2. To serve as a barrier to restrict cellular migration in a selective manner. This is best exemplified by the principles of guided tissue regeneration and guided bone regeneration where nonresorbable polytetrafluoroethylene and resorbable polylactate, polyglycolic acid and calcium sulfate are used.
3. To serve as a scaffold for cellular migration and proliferation. Examples include collagen matrix. Potentially, this scaffold can be further enhanced by selectively defining the types of cells permitted to attach to and proliferate on this matrix with the additions of adhesins and/or integrins.
4. To potentially serve as a time-release mechanism for signaling molecules.

5.1.1 Biomaterials for Growth Factor Delivery

Natural polymers used in bone tissue engineering include collagen, fibrin, alginate, silk, hyaluronic acid and chitosan (CS). Most natural polymers are biocompatible, degradable and readily solubilized in physiological fluid (with exception of chitosan which is soluble under mild acidic conditions), which can be used alone as a growth factor delivery carrier or combined with other delivery materials such as synthetic polymers and inorganic materials. However, natural polymers have several disadvantages such as immunogenicity, difficulty in processing and a potential risk of transmitting animal-originated pathogens (Lee and Shin 2007; Lee et al. 2001).

As a drug delivery carrier, collagen has been fabricated as gels, nanofibers, porous scaffolds and films (Lee and Shin 2007; Lee et al. 2001). Despite the biocompatibility, collagen, like other natural polymers, is mechanically weak and undergoes rapid degradation upon implantation. Therefore, optimization of degradation rate and molecular properties may be required by cross-linking of

collagen with appropriate chemical reagents (Lee and Shin 2007; Lee et al. 2001). Commercially available collagen scaffold materials potentially available for oral tissue engineering application: Collaplug®, Collacote®, Gelfoam® and Helistat® (Kao et al. 2009). The combination of rhBMP-2 delivered in an absorbable type I collagen sponge was approved by the FDA in 2004 as INFUSEs bone graft (Wyeth Pharmaceuticals, Philadelphia, Pennsylvania) for anterior lumbar interbody spine fusion and open tibial shaft fractures and by the European Union (EU) in 2002 as InductOss (Wyeth Pharmaceuticals, Maidenhead, Berkshire, UK) for open tibial shaft fracture. At present, osteogenic protein-1 (OP-1) delivered in a particulate bone-derived type I collagen matrix is available in the USA and the EU as OP-1s implant (Stryker Biotech, Hopkinton, MA) through a Humanitarian Device Exemption (HDE) for recalcitrant nonunion fractures (Chen et al. 2009).

Gelatin is a commonly used natural biomaterial derived from collagen by acid and alkaline processing, and has also received a lot of attention in growth factor delivery systems for periodontal healing when combined with other natural or synthetic polymers (Chen et al. 2005a, 2005b, 2006, 2007a, 2007b, 2009; Takahashi et al. 2007; Pang et al. 2004). In such combinations, gelatin plays a seminal role in modifying the biomaterial properties as well as in influencing their drug release characteristics (Chen et al. 2009).

By virtue of excellent hydrophilicity and tissue biocompatibility, dextrans have been used clinically for more than 70 years for plasma volume expansion, peripheral flow promotion and as antithrombotic agents. Evidence indicates that dextran-based carriers could increase the longevity of therapeutic agents in the circulation, achieved mainly through relatively longer blood half-lives of high M_w dextran conjugates of therapeutic agents, compared with the intact drug or protein (Chen et al. 2009).

A frequently investigated oxide-hydroxide-modified cross-linked dextran is glycidyl methacrylated dextran (Dex-GMA), which can be synthesized by coupling glycidyl methacrylates (GMA) to the hydroxyl functional groups of dextran. Dex-GMA hydrogels are able to control protein release depending upon the network size and their biodegradation characteristics. Drug release from hydrogel microcarriers mainly relies upon swelling of the gel particulates and diffusion of the drugs through aqueous channels. During the initial stages, the hydrogel beads absorb fluid and rapidly enlarge in volume,

culminating in rapid release of the drug through internal particle microtubules to the surface micro-apertures. As the swelling reduces, the drug release slows down and is determined by drug perfusion through the aqueous surroundings and microsphere biodegradation (Chen et al. 2009). Dex-GMA/gelatin scaffolds and scaffold/microsphere composites (Fig. 5.2) have also been generated for applications in periodontal tissue regeneration (Chen et al. 2007a, 2009). Other types of devices employing different configurations from Dex-GMA

and gelatin aimed at the different specific requirements of periodontal defect sites are functionalized bone substitutes loaded with BMP-2, scaffolds fabricated from BMP-2-loaded microspheres and IGF-1-loaded microspheres, and GTR membranes introducing BMP-2-loaded microspheres (Fig. 5.3).

Chitosan (CS) is a deacetylated derivative of chitin, a high molecular weight, second most abundant natural biopolymer commonly found in shells of marine crustaceans and cell walls of fungi. CS is

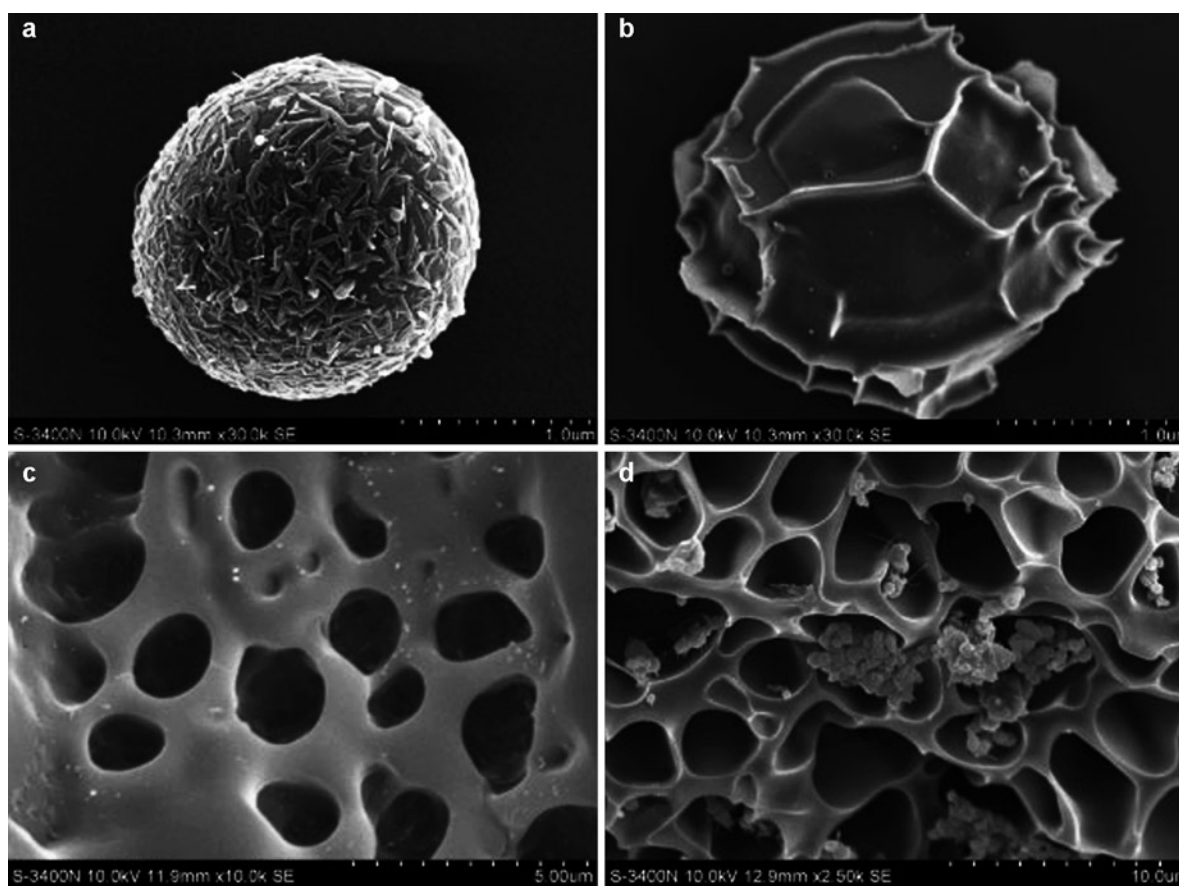


Fig. 5.2 Growth factor delivery vehicles developed for regenerative periodontal therapy: selected representative scanning electronmicrographic images. (a) Freeze-dried glycylmethacrylate derivatized dextran (Dex-GMA)/poly(ethylene glycol) (PEG) microparticles synthesized by Dex-GMA emulsified in an aqueous PEG solution, which were served as bone morphogenetic proteins (BMP)-2 carriers (samples from the same batch of microparticles as shown in Chen et al. (2007a), Fig. 5.3d. (b) Freeze-dried dextran-cogelatin hydrogel microparticles obtained from Dex-GMA and gelatin with corrugated surface served as insulin-like growth factor (IGF)-1carriers, which were fabricated in a so-called aqueous PEG/dextran phase

separation method described in Chen et al. (2006) with minor modification (image from unpublished results). (c) Cutaway view of Dex-GMA/gelatin scaffolds with macroporous pore structure obtained from 5 wt.%PEG, 10 wt.%Dex-GMA, and 5 wt.%gelatin in the polymerizing solution (samples from the same batch of scaffolds as shown in Chen et al. 2007b); detailed fabrication parameters were reported in Chen et al. 2007a). (d) Cutaway view of Dex-GMA/gelatin scaffolds with interconnected pore structure containing Dex-GMA/PEG microparticles loaded with BMP-2 (samples from the same batch of scaffolds as shown in Chen et al. 2009. Reprinted with permission from Elsevier)

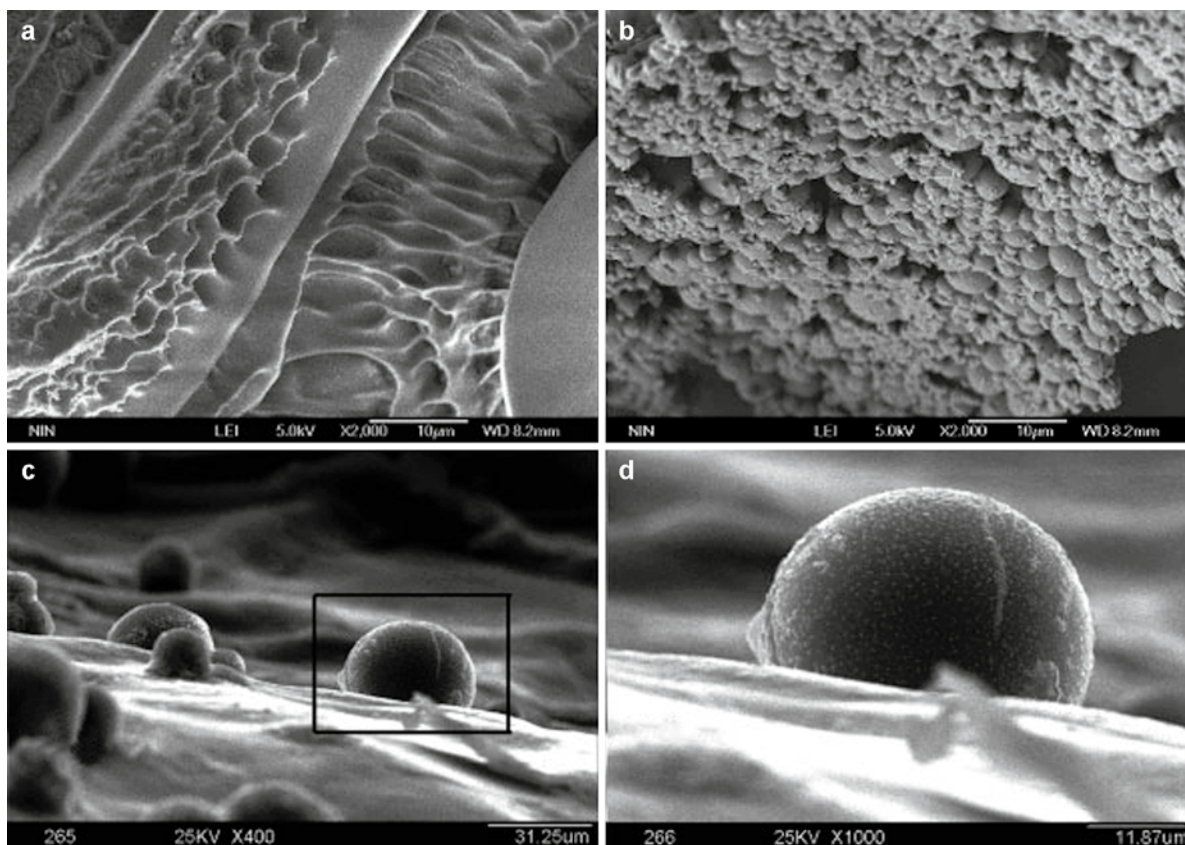


Fig. 5.3 Representative scanning electron micrographic images of growth factor delivery devices (image from unpublished results). **(a)** Functionalized bone substitutes loaded with bonemorphogenetic proteins (BMP)-2 fabricated from glycidylmethacrylate derivatized dextrans (Dex-GMA) and poly(ethylene glycol). **(b)** Scaffolds fabricated from BMP-2-loaded microparticles and IGF-1-loaded microparticles, using the methods recently reported by Jeklenec et al. with minor modification Jaklenec et al. (2008). **(c)** Guided tissue/

bone regeneration membranes for periodontal regenerative therapy obtained from collagen-based biomaterials containing BMP-2-loaded microparticle delivery systems, which were synthesized according to the reported methods with minor modification by Chen et al. (2005). **(d)** Magnified view of the *black rectangle* frame from **(c)**: BMP-2 loaded microparticles were largely immobilized into membranes with integrated configuration (Chen et al. 2009. Reprinted with permission from John Wiley & Sons)

a linear polysaccharide, composed of glucosamine and *N*-acetyl glucosamine linked in a $\beta(1-4)$ manner; the glucosamine/*N*-acetyl glucosamine ratio being referred as the degree of deacetylation. CS-based implants evoke a minimal foreign body reaction, with little or no fibrous encapsulation. One of the properties of CS is that it can be molded in various forms, such as a sponge, porous scaffold and nanofiber. CS also possesses excellent ability to form porous structures. Porous scaffolds are generated by freezing and lyophilizing CS solutions or by processes such as an “internal bubbling process” (IBP) where CaCO_3 is added to chitosan solutions to generate CS- CaCO_3 gels. Another interesting property of CS is its intrinsic

antibacterial activity. Its cationic amino group associates with anions on the bacterial cell wall, suppressing biosynthesis; moreover, CS disrupts the mass transport across the cell wall accelerating the death of bacteria. At present, CS is one of the most promising biopolymers for tissue engineering (Dodane and Vilivalam 1998; Di Martino et al. 2005; Zhang et al. 2010; Muzzarelli 2010).

Perhaps *synthetic polymers* are the most widely used materials as growth factor delivery carriers in tissue engineering. Synthetic materials indeed provide excellent chemical and mechanical properties that natural polymers usually fail to possess. The great advantage of synthetic polymers is associated with their

processibility and flexibility to tailor to have appropriate chemical and mechanical properties. Disadvantages of several synthetic polymers include possible acute and/or chronic inflammatory response, potential localized pH decrease due to relative acidity of hydrolytically degraded by-products, retarded clearance rate and limited biological function. Synthetic biodegradable polymers such as poly(lactic acid) (PLA), polyglycolide (PLG) and their copolymers, poly L-lactic acid (PLLA), poly D,L-lactide-co-glycolic acid (PLGA) and poly ϵ -caprolactone (PCL) are commonly used as carriers of osteoinductive factors (Fig. 5.4) (Lee and Shin 2007; Haidar et al. 2009a). An injectable composite bioresorbable poly(lactic-co-glycolic acid) (PLGA)

and additives forming in situ a matrix designed as a carrier for recombinant human growth/differentiation factor-5 (rhGDF-5) was recently described (Herberg et al. 2008). The PLGA composite showed a highly porous (500–1,000 μm) space-providing structure. It effectively induced coagulation exhibiting an intimate interaction with the fibrin clot. The biphasic biodegradation was complete within 4 weeks. The composite was conveniently injectable (90.4 ± 3.6 N) for ease of use. It exhibited a sustained rhGDF-5 release over 4 weeks (40.8%) after initial burst (3.4%). Sites receiving the composite showed limited, if any, residuals and had no appreciable negative effect on periodontal wound healing. There were no noteworthy

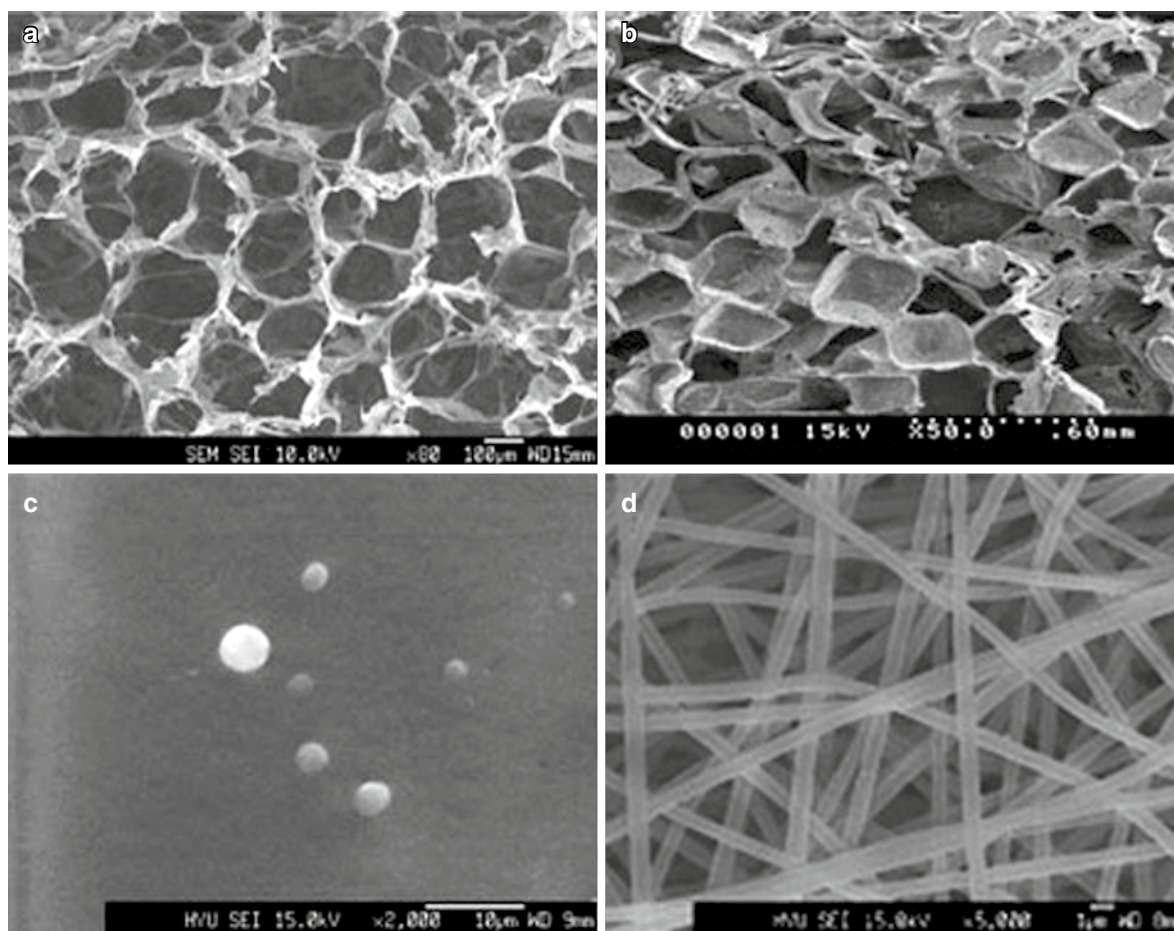


Fig. 5.4 Representative scanning electron micrographic images of growth factor delivery carriers; (a) collagen sponge prepared by a freezing-thawing method, (b) Poly(L-lactic acid) porous scaffold fabricated by a particulate-leaching method,

(c) Poly(lactide-co-caprolactone) microparticles prepared by a double-emulsion-extraction method, and (d) Poly(L-lactic acid) nanofiber fabricated by an electrospinning technique (Lee and Shin 2007. Reprinted with permission from Elsevier)

inflammatory lesions in sites receiving the PLGA composite (Herberg et al. 2008).

Although most growth factor delivery carriers have been produced from natural and synthetic polymers, *inorganic materials* were also used to promote bone formation in specific pharmacological intervention of musculoskeletal problems. In particular, calcium phosphate cements, bioactive glasses, hydroxyapatite and β TCP have been processed to deliver several growth factors such as bone morphogenetic proteins (Lee and Shin 2007; Xie et al. 2010). Among them, calcium phosphate cements possess a great potential as a carrier in bone tissue engineering, highly biocompatible and osteoconductive materials which can stimulate tissue regeneration (Ginebra et al. 2006a, b; Lee and Shin 2007). In general, all calcium phosphate cements are formed by a combination of one or more calcium orthophosphates, which upon mixing with a liquid phase, usually water or an aqueous solution, form a paste which is able to set and harden after being implanted within the body. Most of them form hydroxyapatite upon setting, with low crystallinity and high specific surface, which can incorporate different ions in its lattice depending on the composition of the starting materials. In general, it can be stated that the formation of hydroxyapatite through a cement reaction is a biomimetic process, in the sense that it takes place at body temperature and in a physiological environment. Indeed, the hydroxyapatite formed in the setting of calcium phosphate cement is much more similar to biological apatites than ceramic hydroxyapatite. As mentioned, cement setting is a result of dissolution and precipitation process. The interlocking between precipitated crystals is responsible for cement hardening. After setting, the calcium phosphate cement develops a highly micro/nanoporous structure. Porosity can vary between 30% and 50%, depending on the processing conditions, for example, liquid-to-powder ratio. Unlike calcium phosphate ceramics employed as drug delivery systems, where the drugs are usually absorbed on the surface, in calcium phosphate cements the drugs can be incorporated throughout the whole material volume, by adding them into one of the two cement phases. This fact can facilitate the release of drugs for more prolonged times. Calcium phosphate cements have been used as drug carriers for antibiotics, anti-inflammatory or anticancer drugs, hormones but also growth factors able to stimulate bone regeneration such as bone morphogenetic proteins or transforming growth factors β (TGF- β) (Ginebra et al. 2006a).

Several commercial products are available on the market (Bohner et al. 2005).

5.1.2 Configurations of Growth Factor Delivery Carriers

For the maximization of bone healing efficacy by the localized delivery of osteoinductive factors, it was previously noted that selection of appropriate delivery materials and types of bioactive molecules are crucial. Equal effort should be made to develop strategies how to incorporate growth factors into delivery carriers; immobilization methods may include either noncovalent (physical entrapment, surface adsorption, affinity binding or ionic complexation) or covalent binding (chemical conjugation) to the carrier. The release rate could be controlled by varying the immobilization method. An additional factor determining release kinetics and stability of growth factors is the configuration of the carrier; a delivery system could be designed as *three-dimensional matrices, injectable gels, micro/nanoparticulates and their composites* (Lee and Shin 2007; Chen et al. 2009, 2010) (Fig. 5.5).

For localized growth factor delivery, proteins are most commonly immobilized through *noncovalent or covalent binding to a carrier matrix*. *Noncovalent binding includes physical entrapment, adsorption or ionic complexation*. Bone growth factors have been physically entrapped in polymeric microparticles, liposomes, hydrogels, foams or bone cements. Growth factors have also been dispersed in various types of materials used to coat implant surfaces. Besides physical entrapment, physical adsorption or physisorption of proteins onto implant materials has been frequently used. A third possibility of noncovalent association of a protein with a biomaterial is by ion complexation. Proteins with different isoelectric points (pI) may be used for polyion complexation with charged macromolecules, such as alginate, chitosan, gelatin, hyaluronan and also synthetic polyelectrolytes (Fig. 5.6). As with passive adsorption, problems related to irreversible ion complexation can cause protein inactivation. *Covalent immobilization* is another strategy to retain growth factors for longer periods of time at the delivery site. Covalent immobilization of growth factors was not a priori expected to maintain biological activity, because it may negatively affect their binding to the receptors

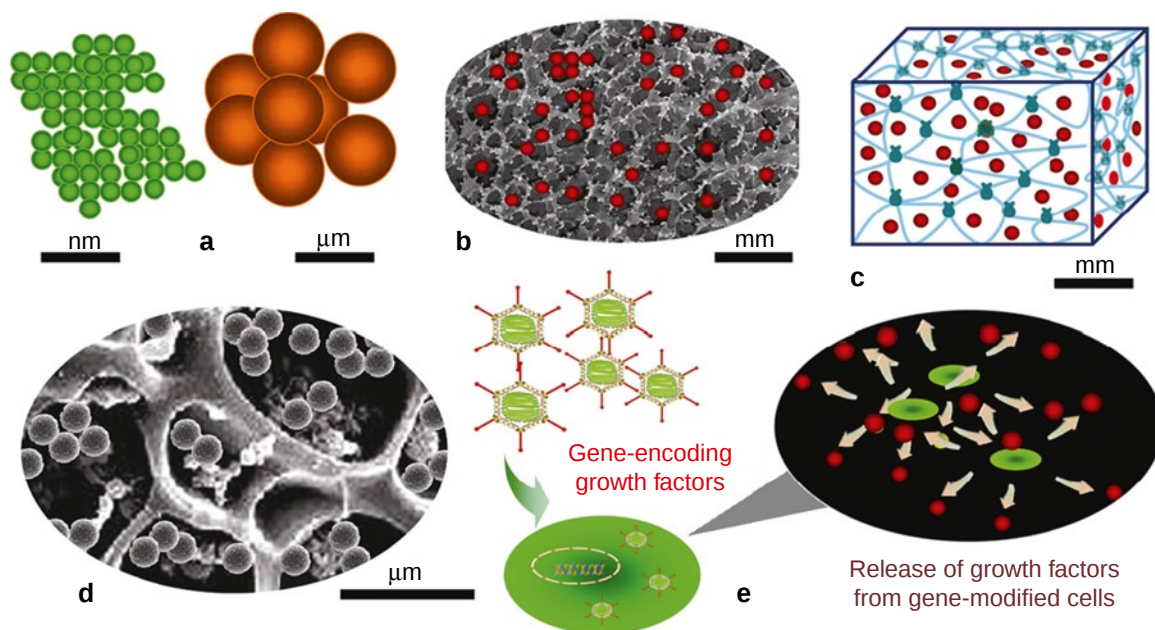


Fig. 5.5 Schematic diagrams of several common samples investigated for the delivery of growth factors (GFs) to the periodontium (Components not scale to actual size). (a) Nanoparticle (green) and microparticle (red) vehicles. (b) GFs immobilized

into a three-dimensional (3D) scaffold. (c) GFs incorporated into hydrogels. (d) GF-loaded particulates incorporated into a polymeric scaffold. (e) Gene delivery for releasing GFs (Chen et al. 2010. Reprinted with permission from Elsevier)

and the subsequent dimerization of the receptors in the plane of the membrane. Nevertheless, if appropriately designed, conjugated growth factors, so-called tethered growth factors, offer important control of the amount and distribution of these components in solid matrices and facilitate the establishment of growth factor gradients (Luginbuehl et al. 2004).

5.2 The Use of Platelet-Rich Plasma (PRP) for Periodontal Regeneration

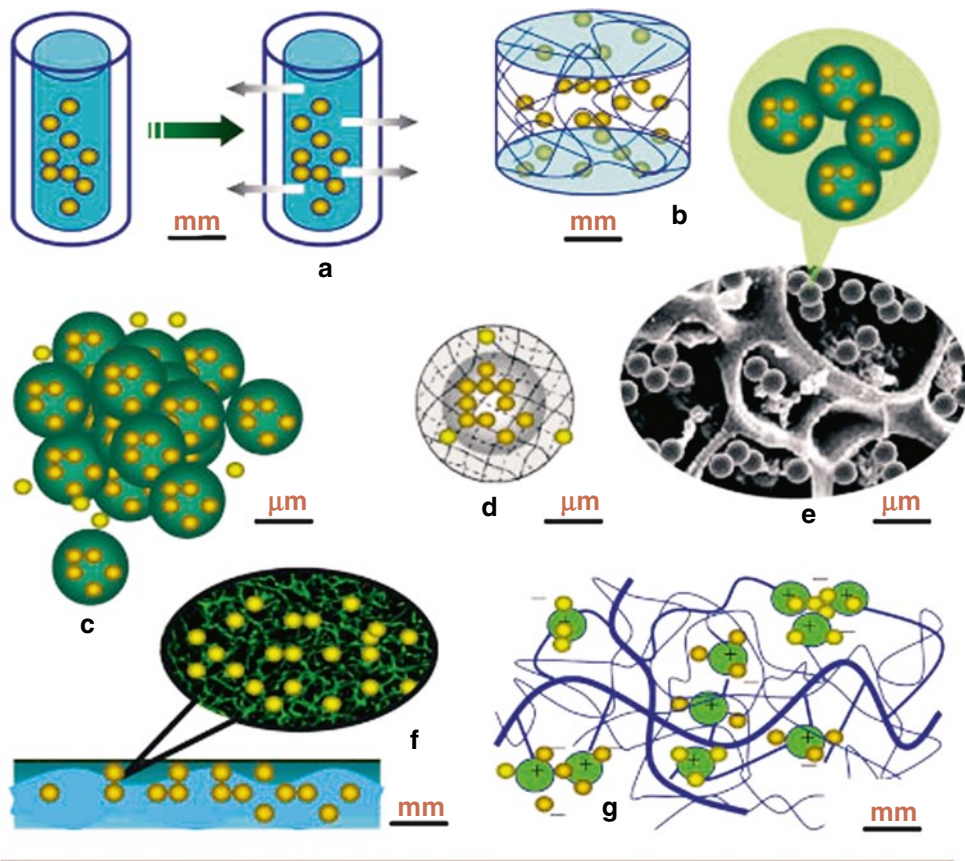
Healing of tissue, both soft and hard, is mediated by a complex array of intra- and extracellular events that are regulated by signaling proteins. Platelets play a prominent, if not deciding, role in this process (Pietrzak and Eppley 2005). Hematoma and clot formation after surgical intervention or trauma initiates the healing cascade. Clot formation is initiated by one of two pathways: intrinsic and extrinsic. The intrinsic pathway is initiated by damage or alteration to the blood itself, whereas the extrinsic pathway is initiated by contact of the blood with factors that are extraneous to the blood

(e.g., damaged tissue). Both pathways involve a cascade of events that, while beginning differently, converge during the latter steps of the process. Platelets and the release of their proteins are essential and necessary for either pathway of clot formation (Mehta and Watson 2008).

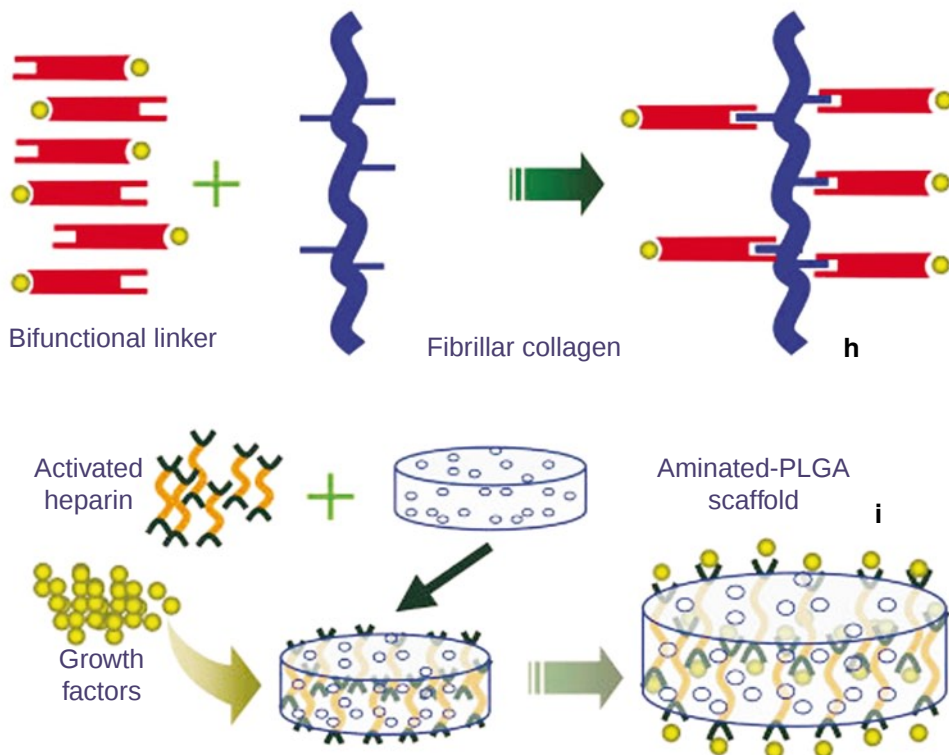
Platelet activation in response to tissue damage and vascular exposure results in the formation of a platelet plug and blood clot to provide hemostasis and the secretion of biologically active proteins. The composition of this naturally occurring hematoma is 95% red blood cells, 4% platelets and 1% white blood cells. However, an analysis of platelet-enriched clot reveals dramatic differences in its composition compared to natural clot with 95% platelets (as opposed to 4%), 4% red blood cells (as opposed to 95% red blood cells) and a similar amount of white blood cells (Mehta and Watson 2008).

Platelet-rich plasma (PRP) is the volume of a plasma fraction of autologous blood that has platelet concentrations above baseline. Platelet-rich plasma can potentially enhance healing by the delivery of various growth factors and cytokines from the α -granules contained in platelets. The basic cytokines identified

Non-covalent strategies



Covalent strategies



in platelets include transforming growth factor- β (TGF- β), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I, IGF-II), fibroblast growth factor (FGF), epidermal growth factor, vascular endothelial growth factor (VEGF) and endothelial cell growth factor. These cytokines play important roles in cell proliferation, chemotaxis, cell differentiation and angiogenesis. A particular value of PRP is that these native cytokines are all present in “normal”

biologic ratios. In contrast, exogenous cytokines such as bone morphogenic protein (BMP) are produced by recombinant technology and are delivered in high doses using a carrier vehicle. Because healing is a highly complex process, there are distinct limitations to the ability of single-factor therapy (i.e., delivery of an exogenous growth factor) to improve tissue healing (Okuda et al. 2003; Foster et al. 2009; Chen et al. 2010) (Table 5.2).

Table 5.2 Growth factors identified within platelet-rich plasma and their physiologic effect

Factor	Target cell/tissue	Function
PD-EGF	Blood vessel cells, outer skin cells	Cell growth, recruitment
	Fibroblasts and many other cell types	Differentiation, skin closure
		Cytokine secretion
PDGF A + B	Fibroblasts, smooth muscle cells, chondrocytes, osteoblasts, mesenchymal stem cells	Potent cell growth, recruitment
		Blood vessel growth, granulation
		Growth factor secretion; matrix formation with BMPs (collagen and bone)
TGF- β 1	Blood vessel tissue, outer skin cells	Blood vessel ($\pm$), collagen synthesis
	Fibroblasts, monocytes	Growth inhibition, apoptosis (cell death)
	TGF gene family includes the BMPs	Differentiation, activation
	Osteoblasts – highest levels of TGF- β r	
IGF-I, II	Bone, blood vessel, skin, other tissues	Cell growth, differentiation, recruitment
	Fibroblasts	Collagen synthesis with PDGF
VEGF, ECGF	Blood vessel cells	Cell growth, migration, new blood vessel growth
		Anti-apoptosis (anti-cell death)
bFGF	Blood vessels, smooth muscle, skin	Cell growth
	Fibroblasts, other cell types	Cell migration, blood vessel growth

Source: Foster et al. (2009). Reprinted with permission from SAGE

PD-EGF platelet-derived epidermal growth factor, PDGF platelet-derived growth factor, BMP bone morphogenetic protein, TGF transforming growth factor, IGF insulin-like growth factor, VEGF vascular endothelial growth factor, ECGF endothelial cell growth factor, bFGF basic fibroblast growth factor

Fig. 5.6 Noncovalent strategies (a–g) and covalent strategies (h) and (i) for growth factor delivery: selected schematic illustrations are considered to have the potential in future periodontal regenerative therapy or have already been investigated as periodontal therapy tools. (a) Reservoir matrix device for growth factor physical entrapment. (b) Sponge matrix for growth factor adsorption. (c) Micro- or nano-sized delivery system for growth factor entrapment. (d) Polymeric coating microcarriers for growth factor binding. (e) Artificial scaffolds containing microparticle growth factor delivery system(s) (the microparticle system can be one type of system loaded with one kind of growth factor, or more than one type

of microparticle system each loaded with one particular growth factor, so as to achieve dual or multiple delivery of growth factors). (f) Absorbable membranes used in guided tissue/bone regeneration for growth factor adsorption and/or entrapment. (g) Charged hydrogels for affinity binding (electrostatic interactions) to take place between hydrogel and growth factor of the opposite charge. (h) Growth factor was covalently cross-linked via activated bifunctional linker to fibrillar collagen. (i) Covalent strategy through the interaction between growth factors and activated heparin conjugated onto the surface of a aminated-PLGA scaffold (Chen et al. 2009). Reprinted with permission from John Wiley & Sons)

5.2.1 Preparation of Platelet-Rich Plasma

By using the common centrifugation technique for separating whole blood in hematology, platelet-rich plasma can be prepared in the operating room during surgery. Depending on the amount of platelet-rich plasma needed, blood is drawn from a large or a small peripheral vein of the patient. The whole blood is treated with citrate-phosphate-dextrose to prevent coagulation. Differential centrifugation is achieved using a first “hard spin” that separates the platelet-poor plasma from the red blood cells and platelet-rich plasma. The second “soft spin” then separates red blood cells from the platelet-rich plasma. The blood components with the highest specific gravity are located on the bottom of the plasma-filled tube. The

platelets are found in a small “pellet” that, before use, must be dispersed evenly in the plasma (Hallman and Thor 2008) (Fig. 5.7).

The regenerative potential of PRP depends on the amount of growth factors and cytokines released when the platelets are activated. Growth factor and cytokine concentration is dependent on the concentration of these proteins in the platelets, the processing technique, which influences platelet concentration and hence protein concentration, and the completeness of platelet activation before measurement. Before assessment of growth factor and cytokine concentration, these proteins must be released from platelets. Platelet activation and growth factor release can be accomplished by the addition of calcium or thrombin to the platelet concentrate (Mehta and Watson 2008) (Fig. 5.8).

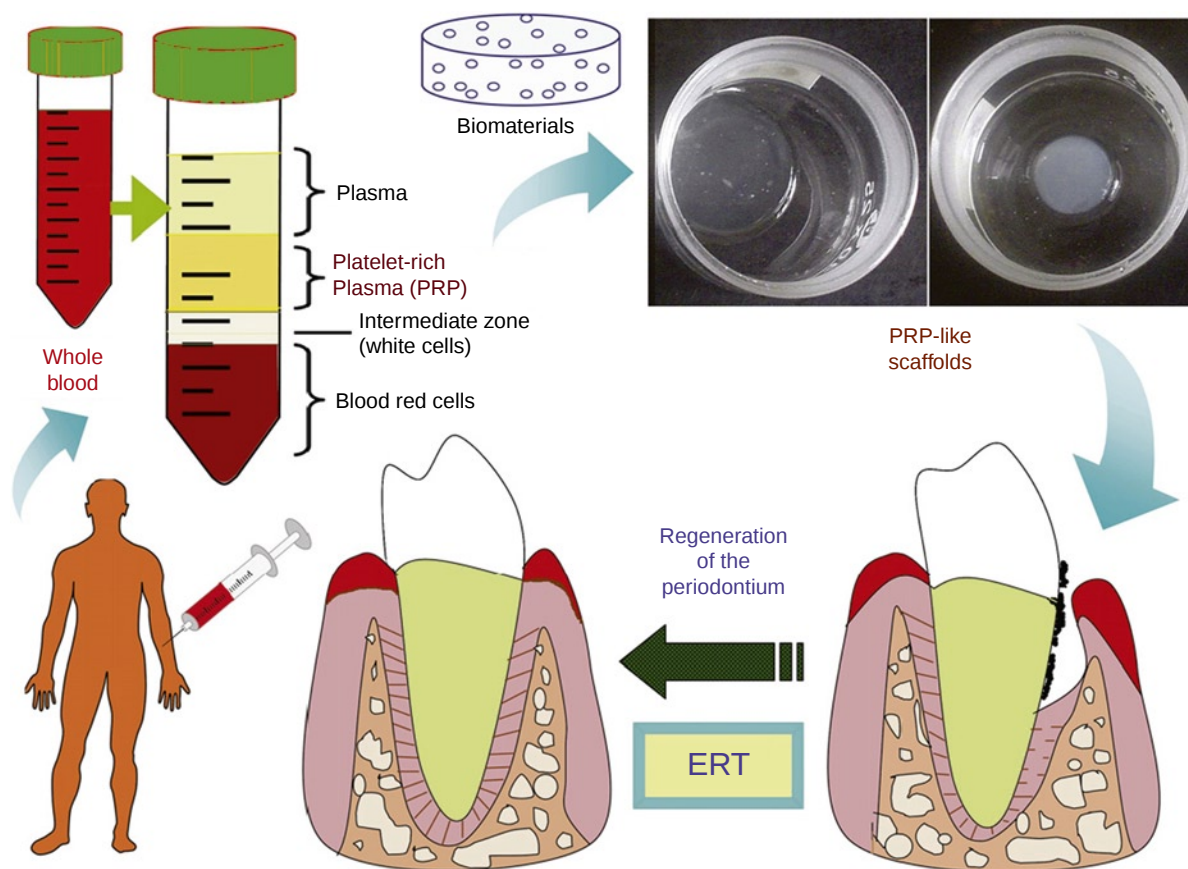


Fig. 5.7 Schematic illustration of the preparation of platelet-rich plasma (PRP)-like scaffolds (gel-like preparations) as a representative endogenous regenerative technology (ERT) for periodontal regeneration (illustration is not to scale). Whole blood was collected and centrifuged according to a strict preparation procedure, for example, plasma/preparation rich in growth factor (PRGF) technology, to generate plasma with the platelets remaining in the bottom of the superior part (the plasma part).

PRP is the fraction of plasma that contained multiple growth factors and could be clotted when calcium chloride was used as clot activator. PRP-like scaffold could be easily obtained by using PRP alone or PRP combined with other biomaterials. When a PRP-like scaffold (with or without containing cells) is ready for use, it can be implanted into periodontal defects for tissue regeneration enhancement (Chen et al. 2010. Reprinted with permission from Elsevier)

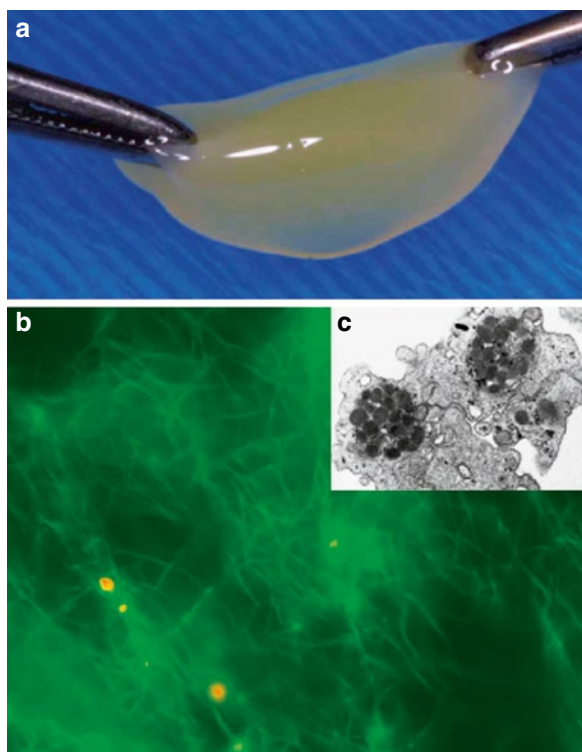


Fig. 5.8 Activation of platelet-rich plasma with calcium brings about thrombin generation and platelet aggregation and the development of a fibrin scaffold. (a) Photograph showing the platelet-rich fibrin scaffold (bar=5 mm). (b) Structure of the platelet-rich fibrin clot as seen by fluorescence microscopy showing a network of fibrin strand (green fluorescence) and platelets aggregates (red-yellow fluorescence) (bar=40 μ m). (c) Transmission electron micrograph of a platelet aggregate showing signs of activation, including the centralization of granules and pseudopod extrusion (arrow) (Anitua et al. 2006. Reprinted with permission from Elsevier)

5.2.2 Handling and Application of Platelet-Rich Plasma

In terms of processing, platelet collection should commence before surgery because activity at the surgical site will initiate clotting, thereby reducing the systemic platelet concentration. It is felt by some that even the initiation of an inhalation anesthetic agent will initiate the activation of platelets. Until the recent development of cell-sensitive filtration systems, centrifugation was the primary basis of producing a platelet-rich fraction. One of the disadvantages of centrifugation is that it can lead to fragmentation and lysis of the platelets, which triggers early release of growth factors and cytokines compromising bioactivity. Fragmentation and early activation have also been shown with previous

filtration-based systems that were either too vigorous or used pediatric dialysis filters in combination with cell saver, resulting in significant platelet degranulation and decreased efficacy. Another disadvantage of centrifugation is that it requires the presence of additional capital equipment in the operating room. In some instances, the nursing or other ancillary staff may be required to transport the patient's blood out of the operating room for centrifugation (Mehta and Watson 2008).

Because the α -granules quickly release their contents on activation, Marx (2004) states that the clotted platelet-rich plasma should be used within 10 min of clot initiation.

This is not an issue with the dual-syringe spray delivery, as the platelet-rich plasma is delivered to the wound site immediately after activation. In the case of other mixing techniques, it is important to transfer the clot to the surgical site before clot retraction; otherwise, the transferred clot may be deficient in the secretory proteins that were expressed (Eppley et al. 2006). Marx et al. (1998) described a protocol for PRP application that requires the use of an individual 10-mL syringe for each mix. Each mix draws, in order, 6 mL of PRP, 1 mL of the calcium chloride/thrombin mix and 1 mL of air to act as a mixing bubble. The syringe is agitated for 6–10 s to initiate clotting. The PRP, now a gel, is added to the graft in several mixes. If several mixes are used, a sterile new syringe is required at each mix. The addition of a small amount of calcium chloride and thrombin from a reused syringe can coagulate the remainder of the PRP in its container. Once the PRP is added to the graft, the fibrin formation binds the otherwise loose cancellous cellular marrow together to assist the surgeon in sculpting the graft. The fibrin network established in the graft is thought to assist the osteoconduction component of bone regeneration (Marx et al. 1998). The therapeutic administration of PRP extends to the treatment of multiple musculoskeletal disorders and to the regeneration and healing of a wide range of tissues (Anitua et al. 2006) (Fig. 5.9).

Man et al. (2001) proposed a technique in which the circulating nurse, using a long, blunt cannula supplied in the processing disposables kit, aspirated 15 cc of platelet-poor plasma into a 20 cc syringe. This was done for all the processing vessels, yielding a total of 30 or 60 cc of plasma, depending on whether 90 or 180 cc of whole blood was processed. The circulating nurse transferred the total volume of plasma into a prelabeled sterile cup located on an instrument tray in the sterile field. The remaining product, approximately 7.5 cc of platelet-poor

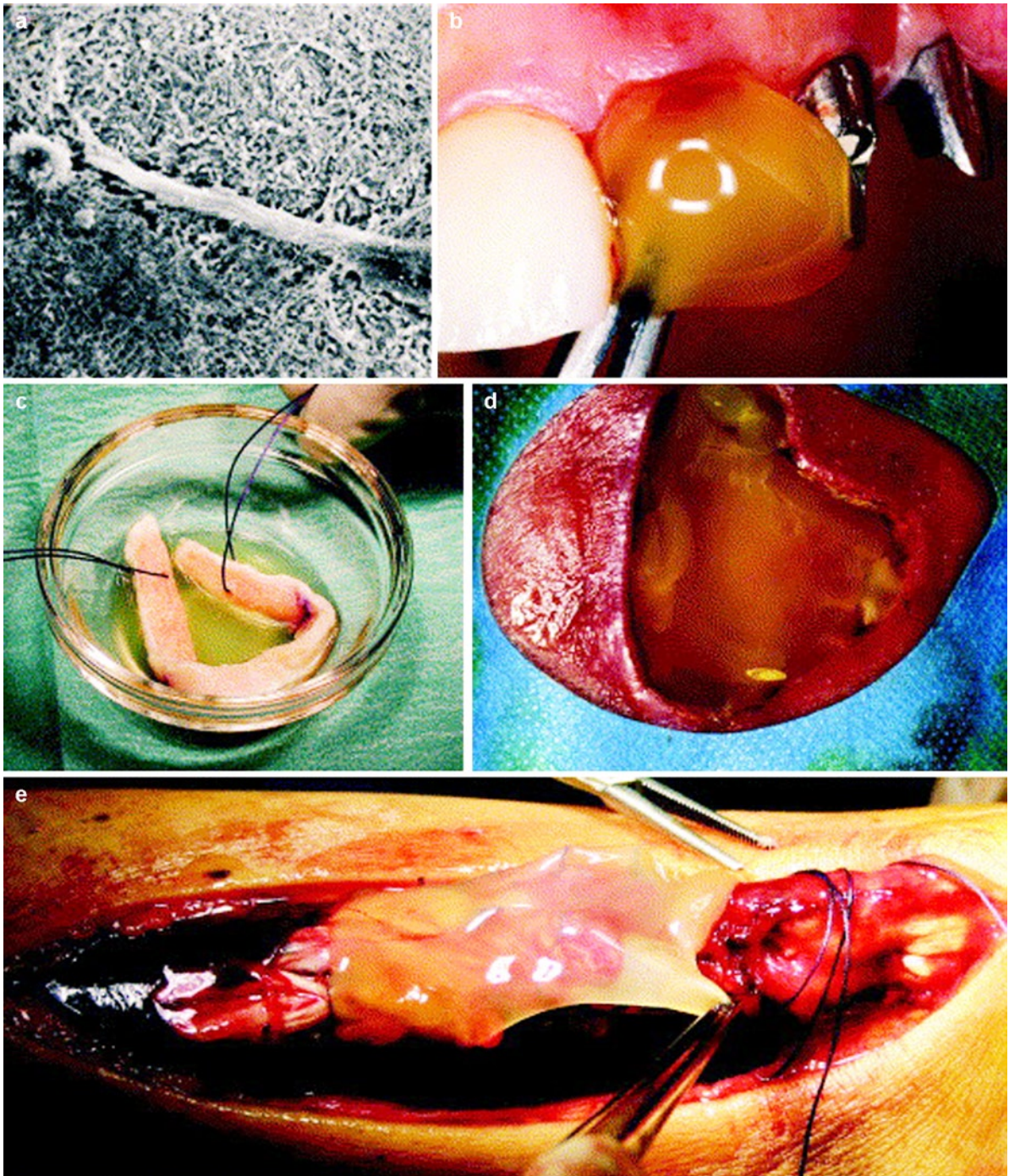


Fig. 5.9 Preparations rich in growth factors (PRGF) are used in a variety of therapeutic applications. (a) One of the roles of fibrin is to promote cell adhesion; scanning electron microscopy reveals osteoblast filopodia on the surface of titanium implants bioactivated with PRGF. (b) Dental implantology: platelet-rich fibrin applied to a post-extraction alveolus so as to enhance bone

regeneration. (c) Transfer of autologous growth factors to a tendon graft used to reconstruct an anterior cruciate ligament. (d) Treatment of a necrotic skin ulcer with PRGF. (e) Surgical repair of an Achilles tendon rupture assisted with PRGF (Anitua et al. 2006. Reprinted with permission from Elsevier)

plasma and the platelet concentrate button, was then mixed to yield platelet-rich plasma. Next, the circulating nurse prepared a thrombin-calcium chloride solution using 5,000 units of thrombin dissolved in 5 cc of 10% calcium chloride. The solution was then transferred by syringe into a sterile cup on the instrument tray in the sterile field. The platelet-poor plasma and calcium thrombin solution was then available for the scrub nurse to use in preparing autologous fibrin glue when needed during the procedure. The preparation of the fibrin glue requires a 10:1 ratio of platelet-poor plasma to thrombin-calcium chloride solution. The easiest method to deliver the plasma and thrombin product is by dual syringe with the platelet-poor plasma drawn into a 10-cc syringe and the thrombin-calcium solution into a 1-cc syringe. The two syringes are then connected to a dual spray applicator tip and then sprayed onto the surgical bed. The platelet-poor plasma and thrombin-calcium cannot be combined before being applied in that they gel almost instantaneously, and their application is very difficult once they have gelled. The dual syringe method allows both solutions to be mixed as they are administered into the surgical bed. This same dual syringe method is also used for the application of autologous platelet gel, simply substituting platelet-rich plasma for platelet-poor plasma (Man et al. 2001).

However, a method of producing small amounts of PRP has recently become available commercially (PRP Kit, Fa. Curasan, 63801 Kleinostheim, Germany). This method seems to have a higher acceptance by patients and it is also less expensive (~€10) (Weibrich et al. 2003).

When five techniques of preparation of PRP: University Hospital of Liège technique, Curasan® (www.curasan.de) PRP Kit, Plateltex® (www.plateltex.com), GPS® (www.biomet.com) and RegenLab® (www.regenkit.com) were compared, it was revealed that technique Plateltex® makes it possible to collect the highest concentration of platelets in the smallest volume available (Kaux et al. 2009).

5.2.3 Safety

Because it is an autogenous preparation, PRC is inherently safe and therefore free from concerns over transmissible diseases such as HIV, hepatitis, West Nile fever and Creutzfeldt–Jakob disease. PRC, therefore, is well accepted by patients. PRC use is contraindicated in patients who have preexisting coagulation defects (thrombocytopenia, hypofibrinogenemia or on anticoagulant therapy) or potentially have a hypersensitivity to bovine

products (Mehta and Watson 2008; Grant et al. 2005). In periodontal surgery, the use of PRP was demonstrated to be entirely safe, without causing complications or adverse events; postoperative healing was uneventful (Trombelli and Farina 2008; Kotsovilis et al. 2010).

5.2.4 Human Studies on Platelet-Rich Plasma

Platelet-rich plasma (PRP) is an example of an autologous product that has been utilized and studied since the 1970s. Platelet-rich plasma has been used clinically in humans for its healing properties attributed to the increased concentrations of autologous growth factors and secretory proteins that may enhance the healing process on a cellular level (Foster et al. 2009). The hope is that PRP enhances the recruitment, proliferation and differentiation of cells involved in tissue regeneration.

In the literature, PRP-related products, also known as PRP, platelet-rich concentrate, platelet pellet, platelet gel, autologous platelet concentrate, preparation rich in growth factors and platelet releasate (Marx 2001; Foster et al. 2009), have been studied with *in vitro* and *in vivo* experiments in the fields of orthopedic surgery (Anitua et al. 2004; Sánchez et al. 2009), eye surgery (Anitua et al. 2004), tendon and ligament repair (Anitua et al. 2004), maxillofacial surgery (i.e., sinus floor elevation, alveolar ridge augmentation, mandibular reconstruction, maxillary cleft repair and treatment of extraction sockets) (Oyama et al. 2004; Boyapati and Wang 2006; Hallman and Thor 2008; Plachokova et al. 2008; Arora et al. 2010) and periodontal surgery (Giannobile and Somerman 2003; Plachokova et al. 2008; Bashutski and Wang 2008; Trombelli and Farina 2008; Kotsovilis et al. 2010; Han et al. 2007) to enhance bone and soft-tissue healing by placing supraphysiological concentrations of autologous platelets at the site of tissue damage (Anitua et al. 2004).

Plachokova et al. (2008) reviewed systematically the reported effects of platelet-rich plasma (PRP) on bone regeneration. Differences in treatment effects for periodontal defects in terms of clinical attachment level (CAL) were significant (ranging from 0.8 to 3.2 mm). Unfortunately, no statistical analysis of the data was possible due to the heterogeneity of the studies.

No RCTs are at present available for evaluating the adjunctive clinical effect of PRP in the treatment of intra-osseous or furcation defects (Trombelli and Farina 2008). Case reports/series have suggested a beneficial effect of PRP in periodontal regenerative

procedures (Papli and Chen 2007; Yamada et al. 2006). Papli and Chen (2007) compared the treatment effects of an intralesional graft of PRP to guided periodontal regeneration (GTR) using a bioabsorbable barrier membrane over a 52-week period. Mean PD reduction of 3 ± 1.41 mm (PRP) and 3.6 ± 1.67 mm (GTR), mean REC increase of 0.8 ± 1.01 mm (PRP) and 0.6 ± 1.14 mm (MEM), mean CAL gain of 2.2 ± 1.79 mm (PRP) and 3 ± 1 mm (GTR), mean radiographic bone fill of 3.24 ± 2.85 mm (PRP) and 2.7 ± 1.9 mm (GTR) and mean defect-angle increase of $15.25^\circ \pm 18.21^\circ$ (PRP) and $22.4^\circ \pm 27.3^\circ$ (GTR) were calculated.

Trombelli and Farina (2008) revealed that the use of PRP combined with several types of grafts for the treatment of intra-osseous defects resulted in a substantial CAL gain (Lekovic et al. 2002; Hanna et al. 2004; Okuda

et al. 2005; Czurylszkiewicz-Cyrana and Banach 2006; Ouyang and Qiao 2006; Demir et al. 2007; Yassibag-Berkman et al. 2007; Yilmaz et al. 2007; Döri et al. 2008b). However, when the additional effect of PRP over the graft was evaluated, contrasting results were reported, ranging from a significant enhancement for PRP (Hanna et al. 2004; Okuda et al. 2005; Ouyang and Qiao 2006) to a null effect (Demir et al. 2007; Yassibag-Berkman et al. 2007; Döri et al. 2008b) (Trombelli and Farina 2008). Similar results were reported by Kotsovilis et al. (2010) (studies included: Okuda et al. 2005; Demir et al. 2007; Döri et al. 2007a, 2007b, 2008a, 2008b; Piemontese et al. 2008) (Figs. 5.10 and 5.11).

It was recently demonstrated that both PRP and PRP combined with DFDBA resulted in significant clinical and radiographic improvement in human periodontal

Fig. 5.10 (a) Activated autologous platelet concentrate in a syringe, (b) mixed with β -TCP granules and a few drops of venous blood (Christgau et al. 2006. Reprinted with permission from John Wiley & Sons)

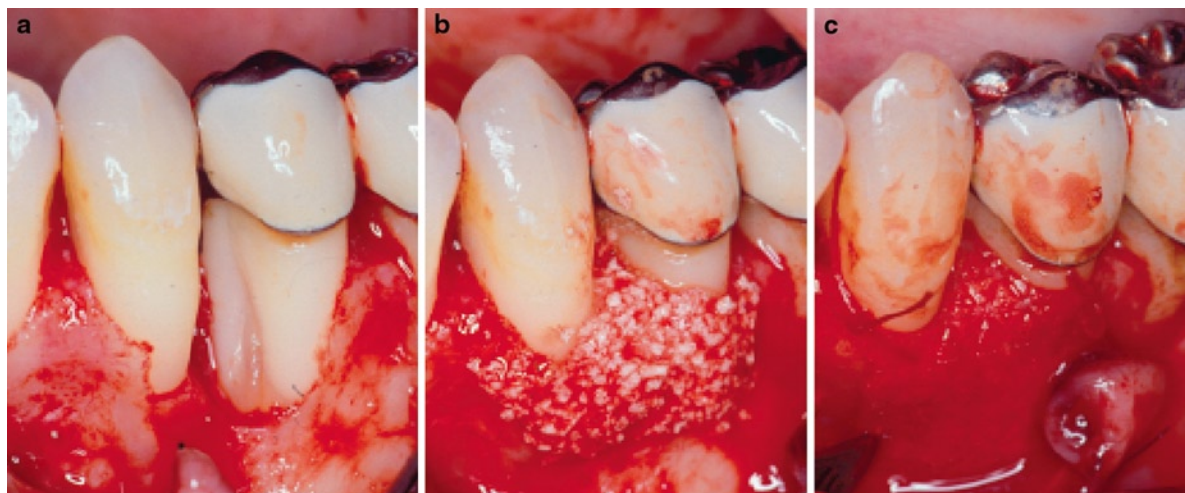


Fig. 5.11 Therapeutic procedure at a mandibular left premolar (test site): (a) after defect debridement, (b) defect fill with TCP soaked in autologous platelet concentrate, and (c) adaptation

and fixation of the bioresorbable guided tissue regeneration membrane (Christgau et al. 2006. Reprinted with permission from John Wiley & Sons)

endosseous defects at 6 months (Markou et al. 2009, 2010), and the addition of DFDBA to PRP did not significantly enhance the treatment outcome (Markou et al. 2009).

A recent randomized, double-masked, clinical trial compared platelet-rich plasma (PRP) combined with a demineralized freeze-dried bone allograft (DFDBA) to DFDBA mixed with a saline solution in the treatment of human intrabony defects. The test group (PRP + DFDBA) exhibited statistically significantly greater changes compared to the control group in probing depth reduction (4.3 ± 1.7 mm vs. 2.6 ± 2.2 mm; $P < 0.05$) and clinical attachment gain (3.5 ± 2.1 mm vs. 2.3 ± 2.4 mm; $P < 0.001$). No statistically significant differences were observed in the hard tissue response between the two treatment groups (Piemontese et al. 2008).

No additional benefit of PRP has been shown when used with graft + GTR over the graft + GTR alone for intra-osseous defects (Christgau et al. 2006; Döri et al. 2007a, 2007b; Trombelli and Farina 2008).

When the current evidence on the role of platelet-rich plasma (PRP) in enhancing root-coverage techniques was reviewed, it has been showed that clinical evidence on the use of PRP in root-coverage procedures is extremely limited. However, available data suggested that the potential benefits of PRP in root-coverage procedures may be improved esthetics, decreased patient morbidity and accelerated wound healing (Bashutski and Wang 2008).

5.2.5 Potential Advantages and Limitations of PRP

If well-controlled clinical trials demonstrate that PRP accelerates the rate of soft tissue injury healing, there are several potential advantages. First, there is a low chance of rejection because the injection is from the patient's autologous blood. Second, PRP can be prepared at the time of care in a simple and relatively inexpensive manner rather than the more complicated process of gathering stem cells (Foster et al. 2009).

There are limitations associated with the use of PRP. An optimal dose range of PRP has yet to be defined. Although application of the PRP may enhance mesenchymal stem cell migration and proliferation, overexposure of cells to PRP may also limit differentiation of those cells into the appropriate cell lines. The review of the literature reveals a rampant lack of standardization in the preparation of PRP, which may in turn have affected

the content of platelets and inflammatory cytokines as well as the contamination of the platelet preparation with leucocytes and erythrocytes. The lack of standardized protocol to produce and evaluate PRP in the literature can help explain the inconsistent clinical and experimental results. To avoid problems in the future, it is imperative to standardize PRP production followed up by a randomized controlled trial to study the effects of PRP on wound healing and promotion of soft tissue and bone healing (Weibrich et al. 2003; Foster et al. 2009; Grageda 2004).

If case reports or prospective clinical trials are to be done in the future, the following standardized protocol is suggested (Grageda 2004):

1. Laboratory test for manual whole blood platelet quantification (baseline).
2. Laboratory test for PRP quantification.
3. Laboratory test for platelet-poor plasma quantification.
4. Commercial enzyme-linked immunosorbent assay (ELISA) laboratory test for quantification of growth factors (PDGF-AB, TGF- β 1, IGF-I) according to the description by Weibrich et al. (2003).
5. Proper histomorphometric analysis of the specimen, according to the model or procedure that was used.
6. The bone graft to be tested with PRP needs to have a contralateral site as a control group.
7. Find the correlation between the histomorphometric analysis and the number of platelets.
8. Find the correlation between the histomorphometric analysis and the number of growth factors.

5.3 Human Platelet-Derived Growth Factor-BB (PDGF)

Platelet-derived growth factor is composed of two polypeptide chains (A and B) and these chains form either a heterodimer or a homodimer. Of the three platelet-derived growth factors (platelet-derived growth factor AB, AA or BB), platelet-derived growth factor BB is biologically most potent (Hammacher et al. 1988; Hollinger et al. 2008). While it was originally identified in platelets, many cell types have subsequently been determined to synthesize platelet-derived growth factor. Reciprocally, many different cell types, particularly those of mesenchymal origin, respond to platelet-derived growth factor. The primary effect of platelet-derived growth factor is that of a mitogen, initiating cell division. In studies using fibroblastic cell types, platelet-derived growth factor has been characterized as a competence

factor. A competence factor classically is a growth factor that makes a cell competent for cell division; a progression factor such as insulin-like growth factor-I or dexamethasone is then necessary to induce mitosis. Thus, in some systems, there is synergy between growth factors of the two groups. However, some cell types respond to platelet-derived growth factor by division without the exogenous addition of other growth factors, perhaps due to autocrine production and stimulation by progression factors (Cochran and Wozney 1999).

In vitro studies suggest that PDGF-BB exerts several important effects on cells native to the periodontal environment (Dennison et al. 1994; Ojima et al. 2003; Boyan et al. 1994; Zaman et al. 1999; Giannobile et al. 2001; Saygin et al. 2000) and *in vivo* preclinical studies suggest that PDGF-BB exerts stimulatory effects on periodontal wound healing and regeneration, alone or in combination with the recombinant insulin growth factor-I (Wang et al. 1994; Cho et al. 1995; Park et al. 1995; Giannobile et al. 1994, 1996; Becker et al. 1992; Lynch et al. 1991a, 1991b, 1989a, 1989b, 1989c; Shirakata et al. 2010), thus motivating clinical follow-up (Lee et al. 2010a) (Fig. 5.12).

A question arises as to why this approach to periodontal and alveolar regeneration is not being aggressively pursued. Several possibilities exist. One reason is

resources. Many studies were funded with private investment dollars, and these sources may have diminished over time. The reason for this is not known but could be attributable to the fact that market approval is a long and costly process for new therapeutic approaches and/or new directions for investors to pursue. Another possibility is that, while periodontal regeneration occurs with exogenous platelet-derived growth factor, the magnitude of the response is not overwhelming compared to existing therapies that the investment in optimizing this approach with carrier molecules, delivery systems etc. is not feasible. A further possibility is that although theoretically platelet-derived growth factor makes sense at the cellular level and can be shown to be effective in animal models, when used in humans with all inherent variables, the efficacy of exogenous platelet-derived growth factor is either difficult to discern, or the effect becomes less obvious due to other factors in the environment. Whatever the reason, this approach to periodontal regeneration was not actively pursued (Cochran and Wozney 1999).

No case report/series or RCTs evaluating the effectiveness of PDGF alone, as applied by a delivery device, in the treatment of intra-osseous and furcation defects, are at present available (Trombelli and Farina 2008). However, one randomized controlled trial assessed the safety of recombinant human (rh) platelet-derived

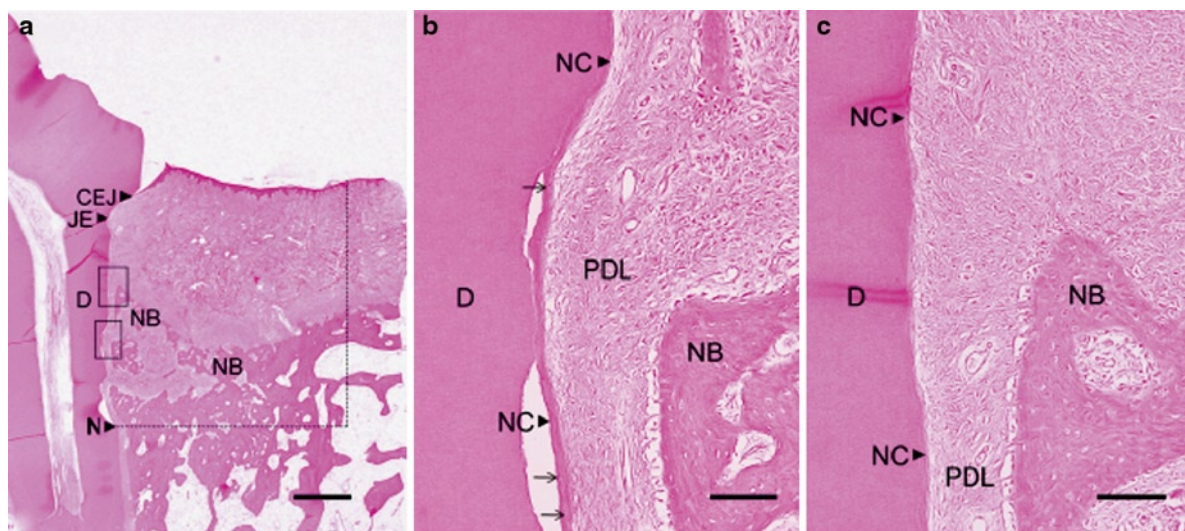


Fig. 5.12 Representative photomicrographs of a two-wall intra-bony defect treated with PDGF/ β -TCP. (a) Overview. New bone (NB) formation is visible extending coronally from the host bone with a slight concaved growth. A small amount of β -TCP and sparse new bone was observed in the middle portion of the defect. The dotted lines show the original defect margin (scale bar: 1 mm; hematoxylin and eosin stain). (b) Higher magnification of the apical framed area in (a). New cellular cementum with inserting collagen fibers was noted predominantly at the

apical portion of the defect. (scale bar: 100 mm; hematoxylin and eosin stain). (c) Higher magnification of the coronal framed area in (a) showing functionally oriented collagen fibers between the new bone and predominantly acellular cementum (scale bar: 100 mm; hematoxylin and eosin stain). CEJ cemento–enamel junction, JE junctional epithelium, NC new cementum, PDL periodontal ligament, N notch (apical extent of root planing), D root dentin, arrows cementocytes (Shirakata et al. 2010). Reprinted with permission from John Wiley & Sons)

growth factor-BB (PDGF-BB) and (rh) insulin-like growth factor-I (IGF-I) when applied to periodontal osseous defects in humans (Howell et al. 1997). Thirty-eight human subjects possessing bilateral osseous periodontal lesions were assigned to one of two treatment groups in a split-mouth design. Following full-thickness flap reflection, test sites received local application of the therapeutic drug delivered in coded syringes by a “masked” investigator. Two dose levels were tested, 50 $\mu\text{g/mL}$ each of rhPDGF-BB and rhIGF-I in a gel vehicle (LD-PDGF/IGF-I) and 150 $\mu\text{g/mL}$ each of rhPDGF-BB and rhIGF-I plus vehicle (HD-PDGF/IGF-I). Control treatment consisted of either conventional periodontal flap surgery or surgery plus vehicle. The primary therapeutic assessment was bone fill measured at reentry 6–9 months after treatment. The results from this study suggest that the local application of rhPDGF-BB and rhIGF-I to periodontal lesions is safe at the dose levels studied. LD-PDGF/IGF-I did not elicit increased defect fill compared to the control; however, HD-PDGF/IGF-I resulted in a significant promotion in

bone regeneration. Additional studies are warranted to more fully characterize the effects of PDGF/IGF-I on periodontal regeneration in humans.

The use of PDGF-BB used in association with an allogenic bone graft (either DFDBA or FDBA) or with β -TCP has shown substantial CAL gain and PPD reduction in case reports on the treatment of class II furcation (Camelo et al. 2003; Nevins et al. 2003) and intra-osseous (Nevins et al. 2003, 2005, 2007) defects. Further studies are needed to determine whether and to what extent rhPDGF-BB graft may be effective for periodontal reconstructive procedures in different periodontal lesions (Trombelli and Farina 2008).

It was showed that when rhPDGF-BB is delivered to promote periodontal tissue engineering of tooth-supporting osseous defects, there is a direct effect on pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen (Sarment et al. 2006) and on growth factors released from the wound (Cooke et al. 2006) (Fig. 5.13). Vascular endothelial growth factor is induced during wound repair, whereas exogenous

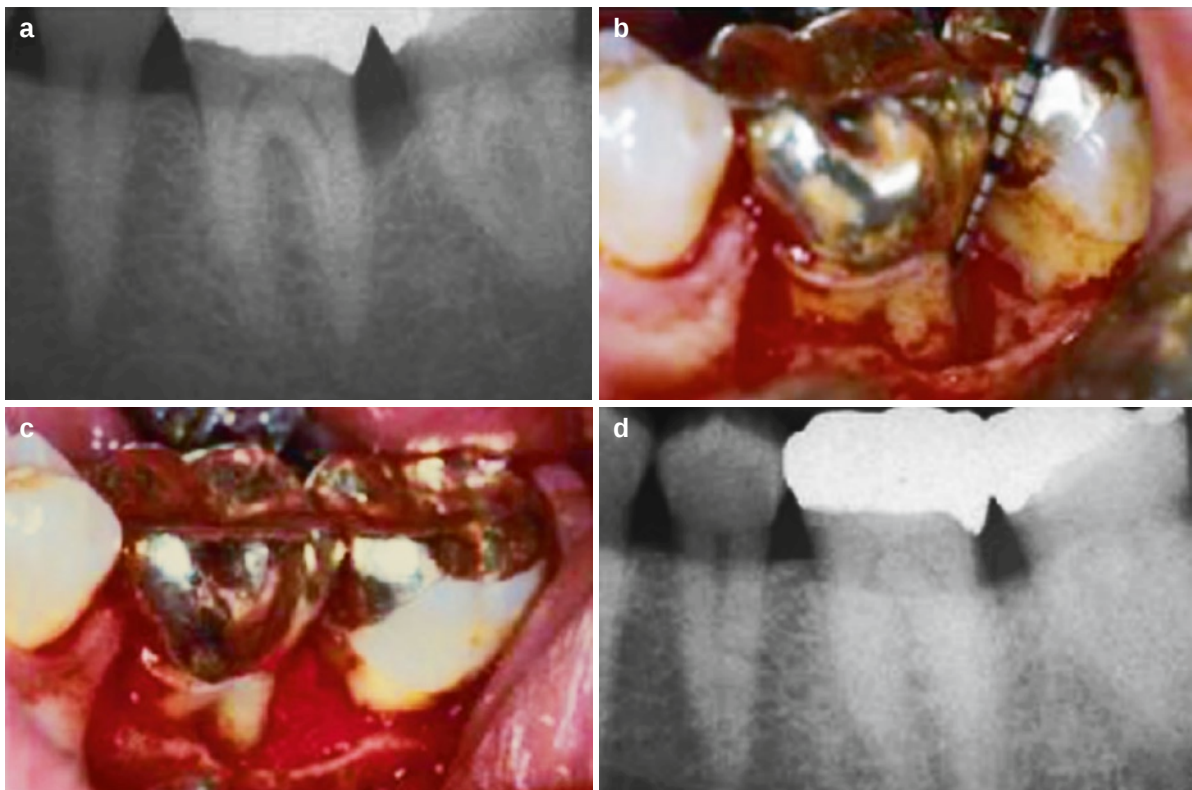


Fig. 5.13 Pretreatment, surgical procedure, and week 24 radiographs of a defect treated with the local delivery of 0.3 mg/mL of rhPDGF and β -tricalcium phosphate. (a) Pretreatment radiograph shows bony defect on the mesial of tooth #18. (b) After flap reflection and degranulation, an infrabony defect of 6 mm is

present. (c) Bone graft placed into infrabony defect. (d) Week 24 postoperative radiograph shows the healing of the infrabony defect. The defect shows 48% bone fill and the crestal lamina dura intact (Sarment et al. 2006. Reprinted with permission from John Wiley & Sons)

PDGF-BB may reduce the release of endogenous PDGF-AB from the wound site after several days of healing. It also appears that there may be a marked increase in bone turnover during the first few days of wound healing when PDGF-BB is added to the osteoconductive scaffold, because the amount of ICTP release from the wound was increased for the 0.3 mg/mL PDGF-BB group (Cooke et al. 2006).

Recombinant human platelet-derived growth factor in combination with a tricalcium phosphate carrier is now commercially available (Kao et al. 2009). The manufacturer of a commercially available growth factor-enhanced matrix (GEM-21S®, Osteohealth, Shirley, NY, www.osteohhealth.com) recommends the combination of 0.5 mL solution (0.3 mg/mL) of rhPDGF-BB with 0.5 g β -tricalcium phosphate (TCP) particles (0.25–1 mm) as vehicle carrier (Schwarz et al. 2009). A large-scale, prospective, blinded and randomized controlled clinical trial recently assessed the safety and effectiveness of purified recombinant human platelet-derived growth factor (rhPDGF-BB) mixed with a synthetic β -tricalcium phosphate (β -TCP) matrix for the treatment of advanced periodontal osseous defects at 6 months of healing. Subjects, each requiring surgical treatment of a 4 mm or greater intrabony periodontal defect, were randomized into one of three treatment groups: (1) β -TCP + 0.3 mg/mL rhPDGF-BB in buffer, (2) β -TCP + 1.0 mg/mL rhPDGF-BB in buffer, and (3) β -TCP + buffer (active control). The study demonstrated that the use of rhPDGF-BB + β -TCP was safe and effective in the treatment of periodontal osseous defects. Treatment with rhPDGF-BB + β -TCP stimulated a significant increase in the rate of CAL gain (3.8 versus 3.3 mm; $P=0.032$), reduced gingival recession at 3 months post-surgery, significantly greater linear bone gain (2.6 versus 0.9 mm, respectively; $P<0.001$) and percent defect fill (57% versus 18%, respectively; $P<0.001$) as compared to a β -TCP bone substitute alone at 6 months (Nevins et al. 2005).

Recently, recombinant human platelet-derived growth factor-BB [from a GEM-21® kit (Osteohealth, Shirley, NY)] was used in conjunction with freeze-dried bone allograft and a barrier membrane to augment both hard and soft tissues simultaneously in preparation for implant placement. Histologic analysis revealed

bone regeneration. Although this approach needs further investigation, this chapter emphasizes the potential for the use of rhPDGF for simultaneous soft and hard tissue implant site preparation (Fagan et al. 2008).

5.4 Peptide P-15

A unique regenerative product is *PepGen P-15*® (Dentsply Friadent, Mannheim, Germany), a calcined bovine bone (1100°C; hydroxyapatite) coated with a pentadecapeptide (P-15, a part of the sequence of collagen). It is available as granulate with a particle size of 0.25–0.42 μ m and used in dental applications (Tadic and Epple 2004). The amino acid peptide, therefore, mimics type I collagen, the major component of bone matrix, promoting cell attachment, which may enhance osteogenesis, acting as an effective substitute for autogenous bone grafts (Precheur et al. 2007; Reynolds et al. 2010). P-15-coated anorganic bone matrix (ABM/P-15) has the ability to promote attachment of human fibroblasts toward the root surfaces, suggesting that P-15-coated ABM may be a useful matrix for bone repair (Qian and Bhatnagar 1996; Lallier et al. 2003). The ABM/P-15 has also the ability to enhance the regenerative capacity of PDL by regulating specific gene expressions of cells during early wound healing (Emecen et al. 2009; Lin et al. 2008). Recently, Lindley et al. (2010) investigated the efficacy of ABM/P-15 treatment in a rabbit model of long bone cancellous healing. Histomorphometric analyses indicated that defects treated with ABM/P-15 had significantly larger areas of new bone formation and significantly more bone growth than defects left empty or filled with ABM. Furthermore, histological examination did not reveal acute inflammatory infiltrate cells in any of the treatment conditions. O'Brien and Blaha (2000) have demonstrated that P-15 can induce ectopic osteogenesis, while Suaid et al. (2010) revealed the regenerative potential of ABM/P-15 in class III furcation defects in dogs. Histologic analysis showed granules from the bone graft surrounded by immature bone matrix and encircled by newly formed tissue. ABM/P-15 putty showed osteoconductive and biocompatible qualities in treatment of intrabony and furcation defects in dogs (Artzi et al. 2006; Roriz et al. 2006). Human histology

showed evidence of periodontal regeneration (new cementum, bone and periodontal ligament) of ABM/P-15 in the treatment of periodontal infrabony defects. Graft particles were still present at 6 months, but no evidence of root resorption, ankylosis or untoward inflammation was seen (Yukna et al. 2002a).

Several clinical studies have provided data regarding the efficacy of this product in the treatment of periodontal defects (Yukna et al. 1998, 2000, 2002b;

Matos et al. 2007; Barros et al. 2006; Radhakrishnan and Anusuya 2004; Bhongade and Tiwari 2007) (Table 5.3). It was demonstrated that the use of the P-15 synthetic cell-binding peptide combined with ABM yields better clinical results than either ABM alone or DFDBA or open flap debridement in intra-bony periodontal defects (Yukna et al. 1998, 2000b; Radhakrishnan and Anusuya 2004; Bhongade and Tiwari 2007). Although promising, these results need to be

Table 5.3 Studies investigating P-15-coated anorganic bone matrix (ABM/P-15) for periodontal healing/regeneration

Study	No. patients	Study period	Periodontal treatment	Main results
Yukna et al. (1998)	31 patients	6–7 months	1. ABM/P-15 2. DFDBA 3. Debridement	The combination ABM/P-15 grafts demonstrated significantly better mean defect fill of 2.8 ± 1.2 mm (72.3%) versus a mean defect fill of 2.0 ± 1.4 mm (51.4%) for defects treated with DFDBA ($P < 0.05$) and a mean defect fill of 1.5 ± 1.3 mm (40.3%) ($P < 0.05$) for defects treated with DEBR. Relative defect fill results showed 87% positive (50–100% defect fill) responses with ABM/P-15, 58% positive responses with DFDBA, and 41% positive responses with DEBR. There were eight to nine times more failures (minimal response) with DFDBA and DEBR (26–29% frequency) than with ABM/P-15.
Yukna et al. (2000)	33 patients	6–7 months	1. ABM/P-15 2. ABM	It was revealed that the combination ABM/P-15 grafts demonstrated significantly better mean defect fill of 2.9 ± 1.2 mm (72.9%) versus a mean defect fill of 2.2 ± 1.4 mm (50.67%) for defects treated with ABM ($P < 0.05$). Other hard tissue findings showed similar clinically superior results with the use of ABM/P-15. Relative defect fill results showed 81% positive (50–100% defect fill) responses with ABM/P-15 and 67% positive responses with ABM. There were 3.5 times as many optimal results ($\geq 90\%$ defect fill) with ABM/P-15 and twice as many failures (minimal response) with ABM. Soft tissue findings showed no significant differences between treatments.
Yukna et al. (2002)	25 patients	3 years	ABM/P-15	Significant clinical changes for the overall group of bony defects included improvement in mean clinical attachment level from 5.4 mm at surgery to 4.5 mm at the 6-month reentry to 3.8 mm at 3 years. There was also a decrease in mean probing depth from 5.3 mm at surgery to 3.1 mm at the 6-month reentry to 2.9 mm at 3 years. The mean gingival recession changed from +0.1 mm at surgery to 1.4 mm at the 6-month reentry to 0.9 mm at 3 years. All of these differences were at least $P < 0.05$ from surgery to the 6-month reentry, and surgery to 3 years, but were not significant from reentry to 3 years via repeated measures analysis of variance.

(continued)

Table 5.3 (continued)

Study	No. patients	Study period	Periodontal treatment	Main results
Matos et al. (2007)	19 patients	6 months	1. ABM/P-15 hydrogel 2. ABM/P-15 particulate	The experimental group demonstrated a mean bone fill of 3.10 ± 0.85 mm versus 3.09 ± 1.11 mm in the control group, which represented a mean defect fill of 75.0% and 73.7%, respectively. Very similar data were obtained for the mean percentages of defect resolution ($85.8\% \pm 10.7\%$ for the experimental group versus $81.9\% \pm 13.3\%$ for the control group). In both groups, gains in CAL were ~ 3 mm (2.89 ± 1.58 in the experimental group versus 3.41 ± 1.95 in the control group), PD reductions were ~ 4 mm (4.02 ± 1.19 in the experimental group versus 4.19 ± 1.55 in the control group), and changes in gingival recession were -1.13 ± 0.96 mm in the experimental group and -0.75 ± 1.08 mm in the control group. At 6 months, there were no significant differences between the groups for any of the outcome measurements. Clinically significant CAL gains (≥ 4 mm) were obtained in 21.7% of the defects treated with ABM/P-15 in hydrogel versus 46.9% of the defects treated with ABM/P-15 particulate. The mean percentage of clinically significant defect bone fill ($\geq 80\%$) was similar with both replacement grafts (39.1% in the experimental group versus 34.4% in the control group).
Kasaj et al. (2008)	26 patients	12 months	1. ABM/P-15 flow 2. Open flap debridement	In comparison with the baseline data, both the test and control groups showed statistically significant reductions of PD and CAL ($P < 0.001$). At 12 months after therapy, the test group showed a reduction in the mean PD from 7.8 ± 1.6 mm to 3.5 ± 1.0 mm ($P < 0.001$) and a change in mean CAL from 8.5 ± 2.1 mm to 4.6 ± 1.2 mm ($P < 0.001$). In the control group, the mean PD decreased from 7.5 ± 0.8 mm to 4.9 ± 0.7 mm ($P < 0.001$) and the mean CAL changed from 8.2 ± 1.2 mm to 6.4 ± 1.4 mm ($P < 0.001$). The PD ($P = 0.002$) reductions and CAL ($P = 0.001$) gains in the test sites were significantly higher than in the control sites. Six sites (46.2%) in the test group presented CAL gain ≥ 4 mm, while this was seen only in one site (7.7%) in the control group. In the test group, a CAL gain of 1 and 2 mm occurred in three sites (23.1%) as opposed to 10 sites (77.0%) in the control group. A CAL gain of 3 mm was measured in four sites (30.8%) in the test group, while this was seen in two sites (15.4%) in the control group.
Pradeep et al. (2004)	14 patients	9 months	1. Autologous PRP + ABM/P-15 2. Autologous PRP	A combination of PRP plus ABM/P-15 was more effective than PRP alone in the treatment of intrabony defects. A statistically significant difference was observed in all clinical parameters in the test group compared to the control group. Furthermore, spiral computed tomography images revealed significantly greater bone fill in the test group.
Radhakrishnan and Anusuya (2004)	10 patients	6 months	1. ABM/P-15 2. Open flap debridement	The combination ABM/P-15 grafts demonstrated significantly better mean defect fill of 3.4 ± 1.7 mm (73.2%) versus a mean defect fill of 0.6 mm (15.8%) for defects treated with OFD. Soft tissue findings showed significant differences among treatments with ABM/P-15 compared to OFD.

Table 5.3 (continued)

Study	No. patients	Study period	Periodontal treatment	Main results
Bhongade and Tiwari (2007)	16 patients	6 months	1. ABM/P-15 putty 2. Open flap debridement	In the test group, the mean CAL at baseline was 6.84 ± 0.28 mm and at 6 months was 3.98 ± 0.25 mm. In the control group, the mean CAL at baseline was 7.08 ± 0.31 mm and at 6 months was 5.66 ± 0.46 mm. The mean CAL gains of 2.86 ± 0.37 mm was observed in the test (ABM/P-15) group, while the control (OFD) group displayed mean CAL gains of 1.42 ± 0.48 mm. The observed differences between baseline CAL and at 6 months were found to be statistically significant in both the groups ($P < 0.05$). The ABM/P-15 treatment resulted in 80% of the defect sites gaining 2.5 mm or more CAL, whereas none of the sites with OFD treatment resulted in a CAL gain of more than 2.5 mm. The mean radiographic DD reduction of 3.4 ± 0.70 mm (70.5%) was observed in the test (ABM/P-15) group, while the control (OFD) group displayed a mean reduction of the defect depth of 0.90 ± 0.61 mm (17.33%) ($P < 0.05$).

ABM anorganic bovine-derived hydroxyapatite bone matrix, DFDBA was compared to demineralized freeze-dried bone allograft, DEBR open flap debridement, PRP platelet-rich plasma

confirmed by further large-cohort, controlled trials specifically designed to assess the relative role of ABM and/or P-15 in the observed clinical improvements (Trombelli and Farina 2008).

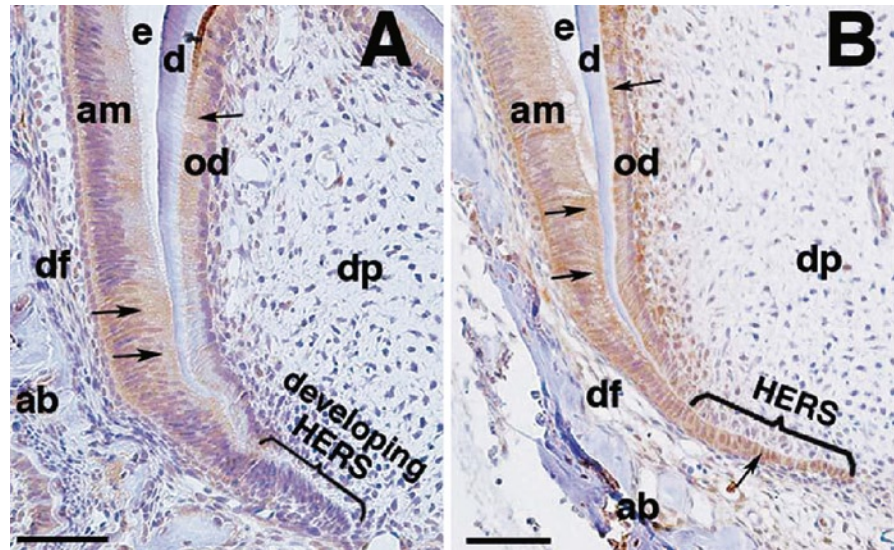
5.5 Insulin-Like Growth Factors

The insulin-like growth factors (IGFs) are a family of mitogenic proteins that control growth, differentiation and the maintenance of differentiated function in numerous tissues. The IGF family includes three ligands (insulin, IGF-I and IGF-II), three cell-surface receptors (the insulin, IGF-I and IGFII/mannose 6-phosphate receptors) and at least six high-affinity IGF-binding proteins (IGFBPs), which bind circulating IGFs and modulate their biological actions (Werner and Katz 2004). Mature IGF-I and IGF-II consist of A, B, C and D-domains. The A- and B-domains of IGFs are homologous to those of insulin. Unlike in the case of insulin, the C-domain is not cleaved off in mature IGFs. IGFs contain an additional D-domain, which is not present in insulin (Duan et al. 2010). In addition to these “classic” family members, more recent work has identified several other proteins as potential components of the IGF system. These “non-classic” members include two additional

receptors (the insulin receptor-related receptor and the insulin-IGF-I hybrid receptor) and a growing number of IGFBPs-related proteins. The emerging role of IGF in oral biology was recently highlighted and their functions in tooth development, growth, PDL homeostasis and different pathological conditions were reviewed by Werner and Katz (2004).

Although IGFs may act as systemic agents, they also are secreted by a number of tissues, including bone, where they are believed to act as local regulators of cell function, such as osteoblast activity and chemotaxis and bone collagen synthesis (Canalis 1992; Strayhorn et al. 1999; Meinel et al. 2003). The periodontal ligament may function as a reservoir for IGFs probably bound to extracellular matrix components (Götz et al. 2006a, 2006b), and there is growing evidence that IGF system plays a very important role in the biology of oro-dento-facial tissues and organs, including the development, homeostasis and regeneration of the periodontium (Fujiwara et al. 2005; Götz et al. 2006a; Sant’Ana et al. 2007; Han and Amar 2003; Termsuknirandorn et al. 2008; Kheralla et al. 2010; Rath-Deschner et al. 2009). IGF-I is involved in early root formation by regulating the mitotic activity in the outer layer of Hertwig’s epithelial root sheath (Fujiwara et al. 2005) (Fig. 5.14). The spatially oriented occurrence of components of the IGF system in human permanent teeth indicates that specific functions of the

Fig. 5.14 Two immunohistochemical localization of the IGF-I receptor α subunit in the cervical region of 3-day-old (a) and 5-day-old (b) mice (arrows positive reaction, am ameloblast layer, ab alveolar bone, d dentin, dp dental pulp, df dental follicle, e enamel, od odontoblast layer, HERS Hertwig's epithelial root sheath). Bars 50 μ m immunoreactivity indicating the presence of the IGF-I receptor was detected in the secretory ameloblasts and odontoblasts (Fig. 5.14a), but not in the epithelial or mesenchymal cells in the cervical region (Fujiwara et al. 2005. Reprinted with permission from Springer)



IGFs may be localized in particular tissue compartments. In the cementum, several IGF components were found indicating roles in tissue homeostasis or attachment. The periodontal ligament (PDL) may function as a reservoir for IGFs probably bound to extracellular matrix components. PDL fibroblasts could then respond in a paracrine manner. In the pulp, the IGF system may be involved in odontoblast biology, fibrosis and denticle formation (Götz et al. 2006a).

In a primate model, the biological effects of platelet-derived growth factor (PDGF) and insulin-like growth factor I (IGF-I) in combination and individually was assessed. At both 4 and 12 weeks vehicle-treated lesions generally revealed minimal osseous defect fill ($8.5 \pm 2.1\%$ and $14.5 \pm 5.7\%$, respectively) and new attachment ($34.1 \pm 5.2\%$ and $26.6 \pm 10.5\%$, respectively). IGF-I treatment did not significantly alter healing compared to vehicle in any parameter at both 4 and 12 week (Giannobile et al. 1996).

Chen et al. (2006) focused on the design of novel hydrogel microspheres based on both dextran- and gelatin-derived biomaterials, and discussed whether locally controlled delivery of IGF-I from dextran-co-gelatin hydrogel microspheres (DG-MP) was useful for periodontal regeneration enhancement in class III furcation defects in dogs. The bone defects received one of the following treatments, randomly distributed among the dogs: (1) blood clot in empty defect (negative control), (2) placebo DG-MP^{6.3} in blood clot (DG-MP control), (3) 100 μ L of IGF-I in blood clot, (4) 100 μ L of IGF-I in DG-MP^{7.8} in blood clot, (5) 100 μ L of IGF-I in DG-MP^{6.3} in blood clot, and (6) 100 μ L of IGF-I in DG-MP^{4.7} in

blood clot. The histological measurement showed significant more tissue regeneration gained in DG-MP groups than in control groups ($P < 0.05$), and at the same time, significant differences were found between three types of DG-MP (DG-MP^{4.7}, DG-MP^{6.3} and DG-MP^{7.8}) groups ($P < 0.05$) (Fig. 5.15). Tissue regeneration in IGF-I in DG-MP^{7.8} group showed statistically more than in other groups ($P < 0.01$). DG-MP groups gained more tissue regeneration than both control groups, and the phenomenon was ultimately demonstrated in following histological measurement and morphometric analysis. DG-MP^{7.8} group also showed the most significant effects ($P < 0.01$). This study has yielded important evidence on bone regeneration in periodontal defects by locally controlled release of IGF-I from dextran-co-gelatin microspheres, and the combined findings strongly suggest that a slow and stable release manner of IGF-I may be more effective on periodontal regeneration (Chen et al. 2006).

Combinations of platelet-derived growth factor and insulin growth factor tested in both animal models (Giannobile et al. 1994, 1996; Lynch et al. 1989c, 1991a, 1991b) and clinical studies (Howell et al. 1997) have demonstrated the ability to enhance periodontal regeneration.

5.6 Fibroblast Growth Factor-2

Fibroblast growth factors (FGFs) that signal through FGF receptors (FGFRs) regulate fundamental developmental pathways, controlling events such as

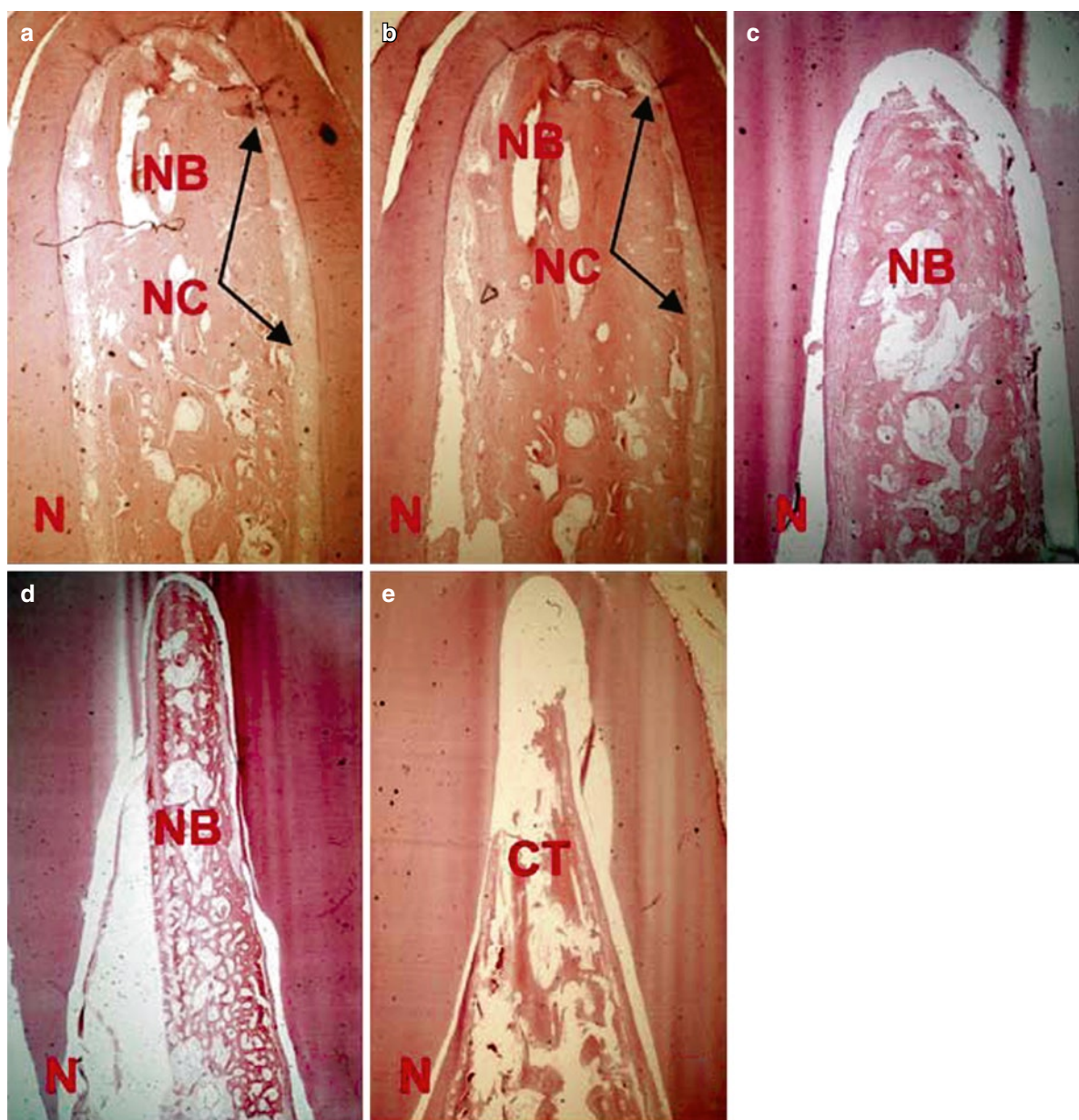


Fig. 5.15 Histological examination of periodontal tissue regeneration (NB new bone, NC new cementum, PDL new periodontal ligaments, N notch, SF Sharpey's fibers, CV capillary vessel, CT connective tissue) in different groups over 8 weeks post-surgery (a–e), the total bone regeneration in the defects, (h) and (e) stained methods, original magnification $\times 12.5$; (f–k) the regeneration of periodontal ligament and newly formed cementum, modified Mallory's trichrome staining methods, original magnification $\times 100$; (l–p) tissue regeneration in the top of the furcation, (h) and (e) stained specimens, original magnification $\times 20$. In IGF-I in DG-MP^{7,8} group, significant amounts of new bone (a) and adequate width of periodontal ligament (f) were observed. The denuded root surface was almost separated the new bone from cementum (f). On the denuded root surfaces of

the furcation area, newly formed cementum covered the surface (l), and Sharpey's fibers inserted into the cementum were frequently observed (f). Complete alveolar bone reconstruction was obtained (a), (f), and (l). In other two IGF-I in DG-MP group, good tissue regeneration but no regular PDL, Sharpey's fibers (G and H) or regular alveolar bone reconstruction (b) and (c), (m) and (n) was obtained. Compared with IGF-I in DG-MP groups, less soft or hard tissue regeneration was observed in the IGF-I in blood clot group (d) and (i). In both control groups, epithelial cells and connective tissue invaded into the top of the furcation in most specimens (e) and (o), and almost no regular PDL and cementum regeneration was observed in the area (d), (e), (j), (k), and (o) (Chen et al. 2006. Reprinted with permission from Elsevier)

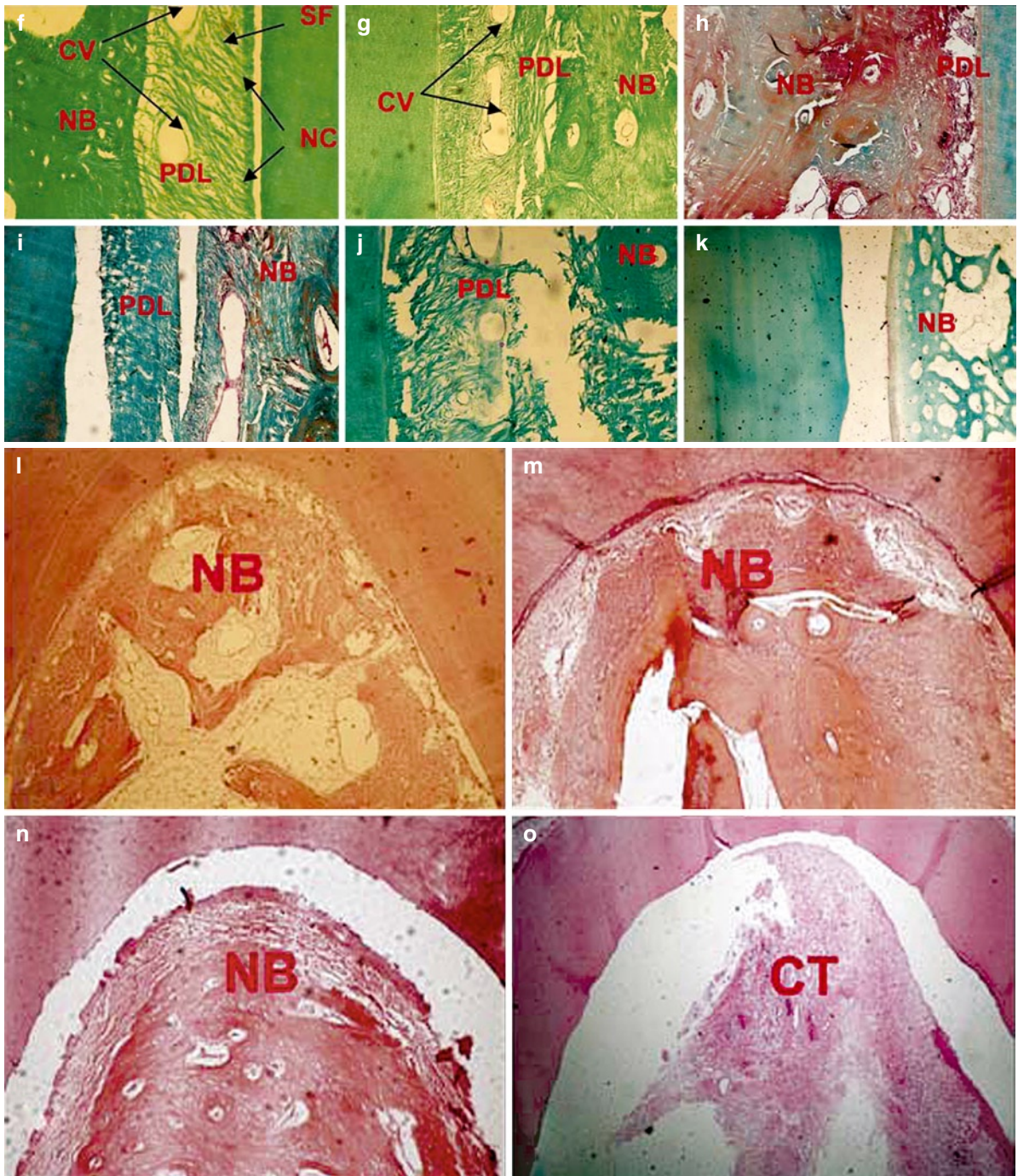


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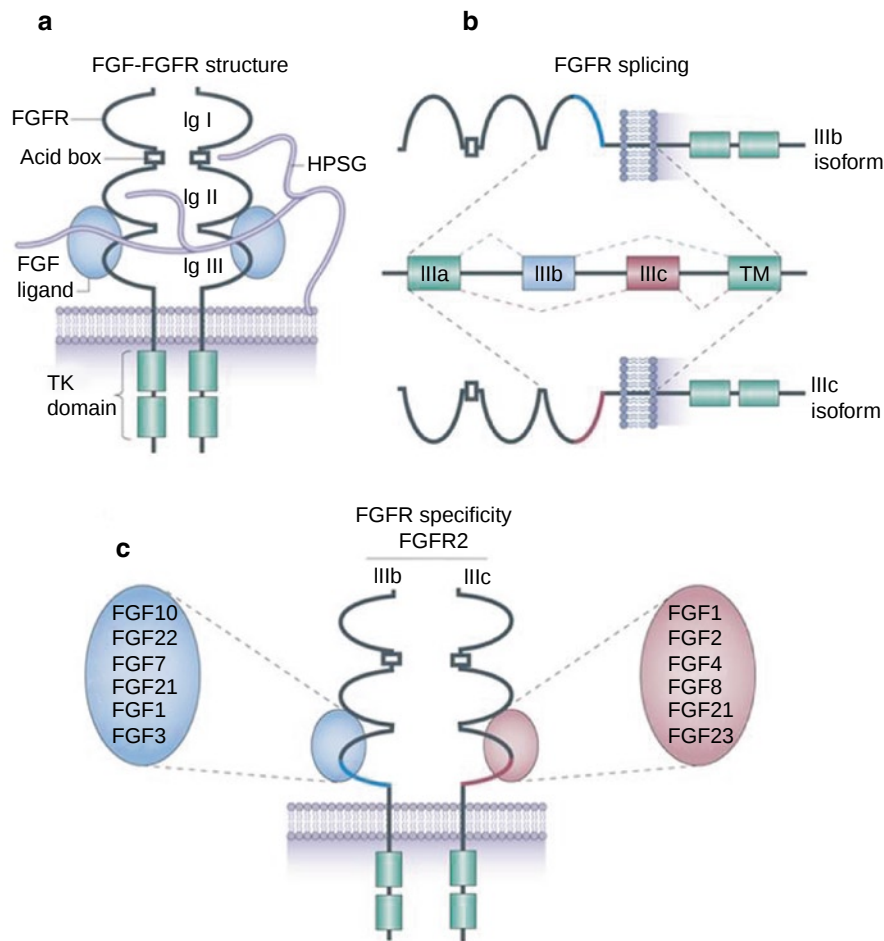


Fig. 5.16 FGFR structure and control of ligand specificity. (a) The basic structure of the fibroblast growth factor (FGF)–FGF receptor (FGFR) complex comprises two receptor molecules, two FGFs, and one heparan sulfate proteoglycan (HSPG) chain. The FGF signaling pathway comprises four highly conserved transmembrane receptors and 18 FGF ligands. FGFs bind with low affinity to cell surface HSPGs (purple) and with high affinity to specific FGFRs. The FGFRs, which are phylogenetically closely related to the vascular endothelial growth factor receptors (VEGFRs) and platelet-derived growth factor receptors (PDGFRs), consist of three extracellular immunoglobulin (Ig) domains, a single transmembrane helix and an intracellular split tyrosine kinase (TK) domain. The second and third Ig domains form the ligand-binding pocket and have distinct domains that bind both FGFs and HSPGs. (b) Ligand-binding specificity is generated by alternative splicing of the Ig III

domain. The first half of Ig III is encoded by an invariant exon (IIIa), which is spliced to either exon IIIb or IIIc, both of which splice to the exon that encodes the transmembrane (TM) region. Epithelial tissues predominantly express the IIIb isoform and mesenchymal tissues express IIIc. FGFR4 is expressed as a single isoform that is paralogous to FGFR-IIIc. (c) Examples of the extent to which ligand specificity can differ between FGFR-IIIb and FGFR-IIIc isoforms, illustrated with the differing ligand specificity of FGFR2 isoforms. The FGFR2-IIIb ligands are shown in blue and the FGFR2-IIIc ligands are shown in brown. For example, FGF7 and FGF10 bind specifically to FGFR2-IIIb and have essentially no binding to FGFR2-IIIc. The mechanisms controlling splice isoform choice are becoming clearer and defined control elements have been identified in the introns surrounding alternatively spliced exons (Turner and Grose 2010. Reprinted with permission from Nature Publishing Group)

mesoderm patterning in the early embryo through to the development of multiple organ systems. FGF signaling extends to many physiological roles in the adult organism, including the regulation of angiogenesis and wound repair. FGFRs are expressed on many different cell types and regulate key cell behaviors, such as proliferation, differentiation and survival, which makes FGF signaling susceptible to subversion by

cancer cells. FGF signaling has evolved to become a highly complex growth factor signaling pathway, reflecting the multitude of physiological functions that are controlled by FGF signaling. The mammalian FGF family comprises 18 ligands, which exert their actions through four highly conserved transmembrane tyrosine kinase receptors (FGFR 1, FGFR2, FGFR3 and FGFR4) (Fig. 5.16). A fifth related receptor, FGFR5

(also known as FGFR1), can bind FGFs, but has no tyrosine kinase domain, and might negatively regulate signaling (Turner and Grose 2010).

Basic fibroblast growth factor (also known as FGF-2) is another growth factor that has been well studied. Fibroblast growth factor-2 is a basic protein originally isolated from hypophysis with an isoelectric point of 9.6 and a molecular weight of 17,000 (Kao et al. 2009). bFGF-2 performs broad mitogenic and cell survival activities, and is involved in a variety of biological processes, including angiogenesis (Gospondarowicz et al. 1986), wound healing (Gibran et al. 1994; Okumura et al. 1996; Richard et al. 1995; Yu et al. 1994), bone growth, development and healing (de Moerloose and Dickson 1997; Kress et al. 2000; Kato et al. 1998) and embryonic development (Tatsumi et al. 2001). bFGF is also an important factor in regulating migration and proliferation of periodontal ligament cells (Murakami et al. 1999; Ge and Yang 2001; Zhang et al. 2001; Ling and Li 2004; Fujita et al. 2004; Tan et al. 2005; Shimabukuro et al. 2005, 2008, 2010; Belal et al. 2006; Silverio-Ruiz et al. 2007; Terashima et al. 2008; Tamura et al. 2008; Sako and Hosomichi 2010; Kanaya et al. 2010; Seshima et al. 2010).

Furthermore, bFGF has been reported to support periodontal regeneration effectively without ankylosis or epithelial downgrowth in intrabony and furcation defects in preclinical models (Fig. 5.17) (Murakami et al. 1999, 2003; Takayama et al. 2001; Rossa et al. 2000; Sato et al. 2004; Shirakata et al. 2010) (Table 5.4). In the defects treated with bFGF, new, dominantly thick, cellular intrinsic fiber cementum and new bone formation were observed. Functionally oriented collagen fibers with many blood vessels in some parts of the denuded root surface were also observed. Interestingly, in the bFGF group, no collapse of the flap was found without carrier materials, and this group showed the greatest amount of new bone formation, may be because the bFGF/hydroxypropyl cellulose applied, prepared as a gel-like material containing 0.3% rhb-FGF, caused rapid and intensive osteogenic bone formation by stimulating the proliferation of osteoblasts rather than that of fibroblasts, vascular endothelial cells or epithelial cells (Fig. 5.18) (Shirakata et al. 2010). It has been showed that β tricalcium phosphate (β -TCP) may be a suitable scaffold for FGF-2 and that the combination of β -TCP and FGF-2 can enhance bone and cementum formation. In a dog model, at 8 weeks following treatment, the β TCP/FGF-2 group

showed a statistically significant increase in both new bone and cementum formation compared to the FGF-2-alone group (76.3% vs. 65.3%, $P < 0.01$; 81.0% vs. 68.3%, $P < 0.01$, respectively) (Oi et al. 2009).

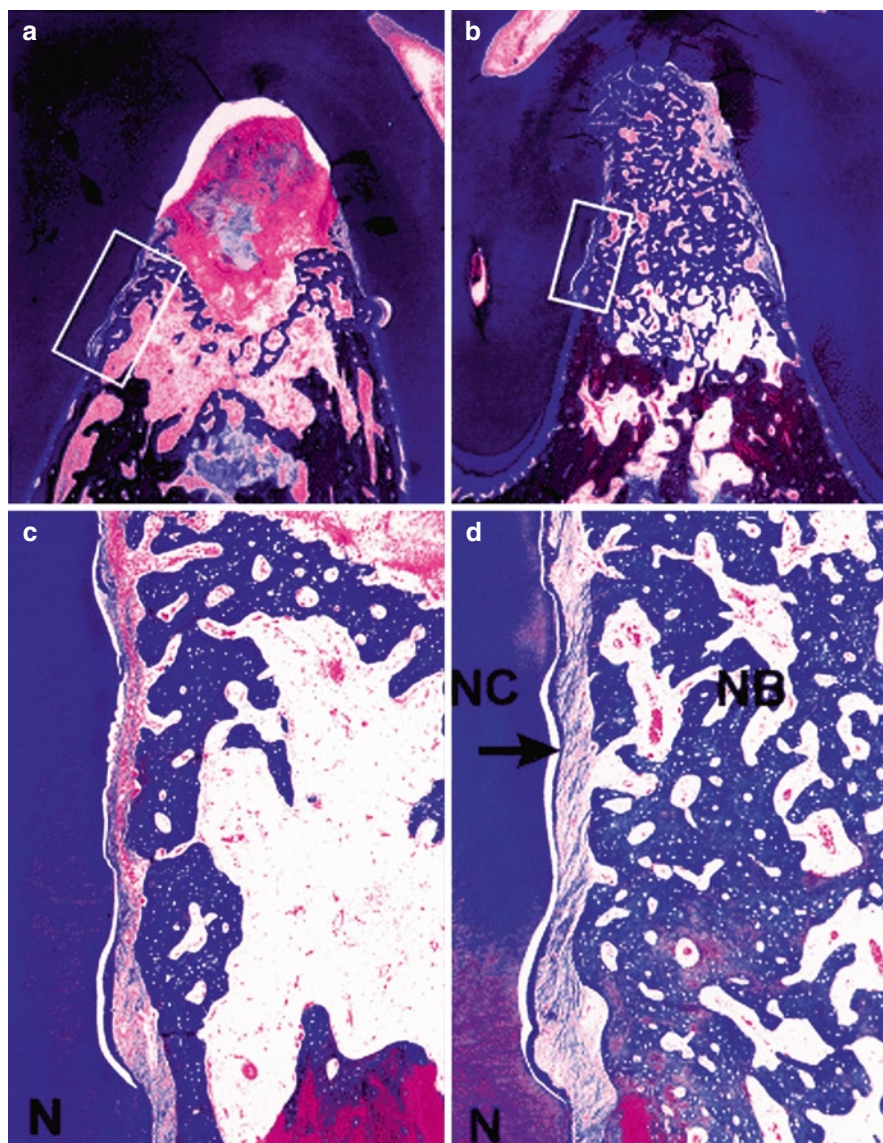
A recent Phase II clinical trial demonstrated that FGF-2 may be effective in regenerating periodontal tissue (Kitamura et al. 2008). The recombinant human FGF-2 with 3% hydroxypropylcellulose (HPC) as vehicle was used in treating 2- or 3-walled vertical bone defect as measured ≥ 3 mm apical to the bone crest. Patients were randomly assigned to four groups: Group A: placebo control; Group B: given 0.03% FGF-2; Group C: given 0.1% FGF-2 and Group D: given 0.3% FGF-2. 0.3% FGF-2 improved CAL by about 2 mm at 9 months from base. And more importantly, rate of increase in bone height observed in close proximity to the dental root was significantly improved in 0.3% FGF-2 treatment group (58.62%) compared to placebo group (23.92%) at 36 weeks ($P = 0.021$) (Fig. 5.19). No adverse effects were observed during the course of this multicenter trial. This finding suggests that topical application of fibroblast growth factor-2 can be efficacious in regenerating periodontal tissue of patients with 2-walled or 3-walled intrabony defects.

No clinical studies evaluating the effect of the association of FGF with grafts and/or GTR in the treatment of intra-osseous or furcation defects are at present available (Trombelli and Farina 2008).

5.7 Transforming Growth Factor- β

TGF- β is synthesized by many tissues, but bone and platelets are the major source of this cytokine. TGF- β is a polypeptide that stimulates the proliferation of osteoblast precursor cells, and it has direct stimulatory effects on bone collagen synthesis. Therefore, TGF- β modulates bone matrix synthesis by increasing the number of cells capable of expressing the osteoblast genotype, as well as direct upregulation of osteoblasts. TGF- β also decreases bone resorption by inducing apoptosis of osteoclasts. In addition to osteoblasts, TGF- β activates fibroblasts to induce collagen formation, endothelial cells for angiogenesis, chondroprogenitor cells for cartilage and mesenchymal cells in an effort to increase the population of wound healing cells (Mehta and Watson 2008).

Fig. 5.17 Histologic overview of furcation class II bone defects in the mesio-distal plane at 6 weeks after application of experimental materials. Representative histologic overviews of furcation defects in the carrier-applied sites (a) and (c) and bFGF-applied sites (b) and (d) are shown. Higher magnifications of panels (a) and (b) ($\times 25$) are shown in panels (c) and (d) ($\times 50$), respectively. The base of the defects is marked by notch (N) in each panel. New cementum (NC) with Sharpey's fibers could be observed on the instrumented root surface. The newly formed periodontal ligament fibers were functionally oriented, and new connective tissue fibers were inserted into both the new cementum and new bone (NB) (Murakami et al. 2003. Reprinted with permission from John Wiley & Sons)



TGF- β may play an important role in the modulation of tissue formation and development of the periodontium (Gao et al. 1998; Wise et al. 1992), is effective in inducing fibroblastic differentiation of PDL stem/progenitor cells and stimulates periodontal ligament cell extracellular matrix synthesis, mitogenesis and proliferation (Fujii et al. 2010; Matsuda et al. 1992; Dennison et al. 1994; Nishimura and Terranova 1996; Lu et al. 1997; Sant'Ana et al. 2007; Silverio-Ruiz et al. 2007; Rodrigues et al. 2007; Okuda et al. 2003).

It was also suggested that TGF- β stimulates wound healing (Okuda et al. 1998; Momose et al. 2002).

Upregulation of the cytokine in inflamed gingiva may counterbalance for destructive gingival inflammatory responses that are simultaneously taking place in patients with chronic periodontitis (Steinsvoll et al. 1999). Elevated levels of TGF- β have been detected in gingival crevicular fluid of patients with gingivitis, periodontitis (Skaleric et al. 1997; Steinsvoll et al. 1999; Mogi et al. 1999; Wright et al. 2003; Alpagot et al. 2008; Gürkan et al. 2006) or gingival overgrowth (Ellis et al. 2004; Wright et al. 2004; Gürkan et al. 2008). It seems also that TGF- β 1 is capable of mediating periodontal regeneration (Parkar et al. 2001; Soory

Table 5.4 Studies investigating bFGF for periodontal healing/regeneration: selective data from animal experiments

Study	Platform	Periodontal defect treated	Carrier	Study groups	Healing interval	Main results
Murakami et al. (1999)	Dogs and monkeys	3-wall intrabony defects and furcation class II bone defects	Fibrin gel or gelatinous carrier	1. Carrier alone 2. Carrier/bFGF	2, 4, 6, 8 weeks	Considerable amounts of new bone formation were observed in 3-wall periodontal defects 2 weeks after bFGF application. After 4 weeks, significant periodontal ligament formation with new cementum deposits and new bone formation were observed in all sites where bFGF was applied in greater amounts than in the control sites. In the control group, bone and cementum regeneration was relatively limited. When bFGF was applied to furcation class II defects of naturally occurring periodontitis in dogs, it was observed significant periodontal regeneration with 79.6% new bone formation rate (NBR), 41.4% new trabecular bone formation rate (NTBR) and 75.8% new cementum formation rate comparing with the controls were the similar values were 42.8% (NBR), 21.8% (NTBR) and 34.3% (NCR). The bFGF application to furcation class II defects in monkeys, it was observed significant periodontal regeneration with 71.3% of NBR, 48.7% of NTBR and 71.2% NBR compared to 54.3% of NBR, 31.6% of NTBR and 38.9% NBR in control sites. No instances of epithelial down growth, ankylosis or root resorption were observed in the bFGF sites.

Murakami et al. (2003)	Dogs	Furcation class II defects	Gelatinous carrier	1. Carrier alone 2. Carrier/bFGF 0.1%	6 weeks	Topical application of bFGF to artificially prepared furcation class II defects accelerated periodontal regeneration in the most central plane with a new bone formation rate of $83.6 \pm 14.3\%$, an new trabecular bone formation rate of $44.1 \pm 9.5\%$, and an new cementum formation rate of $97.0 \pm 7.5\%$. In contrast, the new bone formation rate, new trabecular bone formation rate, and new cementum formation rate were $35.4 \pm 8.9\%$, $16.6 \pm 6.2\%$, and $37.2 \pm 15.1\%$ in the vehicle-applied sites, respectively. Moreover, no instances of epithelial down growth, ankylosis or root resorption were observed in the bFGF-applied sites examined.
Takayama et al. (2001)	Monkeys	Furcation class II defects	Gelatinous carrier	1. Carrier alone 2. Carrier/bFGF 0.1% 3. Carrier/bFGF 0.4% 4. Untreated	8 weeks	Local applications of FGF-2 enhanced periodontal regeneration in a dose-dependent manner when compared with the control and vehicle-applied sites, with the 0.4% FGF-2-treated sites exhibiting significant regeneration of new bone growth rate ($71.3 \pm 13.5\%$), new trabecular bone growth rate ($48.7 \pm 8.9\%$), and new cementum formation rate ($72.2 \pm 14.4\%$). Although the new cementum formation rate in the 0.1% FGF-2-applied sites was also significantly increased, as compared with the control and vehicle-applied sites, new bone growth rate and new trabecular bone growth rate in these sites were not.

(continued)

Table 5.4 (continued)

Study	Platform	Periodontal defect treated	Carrier	Study groups	Healing interval	Main results
Rossa et al. (2000)	Dogs	Furcation class III defects	Lyophilized bFGF	1. GTR 2. GTR + 0.5 mg of bFGF 3. GTR + 1.0 mg of bFGF	90 days	The descriptive analysis indicated better regenerative results in both groups treated with b-FGF while the histometric data, analyzed by means of analysis of variance (ANOVA), showed greater filling of the defects in group 2 in comparison to the defects in groups 3 and 1, respectively, which was represented by a smaller area of plaque-occupied space ($P=0.004$) as well as a greater amount of newly formed cementum ($P=0.002$).
Sato et al. (2004)	Dogs	Extracted and replanted teeth	Collagen gel (Celligen, Koken, Japan)	1. 0.1 μ m bFGF/carrier 2. 1 μ m bFGF/carrier 3. 5 μ m bFGF/carrier 4. Carrier	8 weeks	Eight weeks post-surgery, formation of cementum on denuded dentin was enhanced by application of 0.1, 1 or 5 μ m of bFGF in a collagen gel compared to collagen gel containing vehicle. Histological analyses revealed that at 4 weeks post-surgery, random periodontal ligament fibers had bound to dentin, but were attached only to denuded dentin to which 0.1, 1 or 5 μ m of bFGF in collagen gel had been applied. At 8 weeks post-surgery, we observed the formation of dense fibers bound to alveolar bone and newly synthesized cementum in teeth treated with 1 μ m of bFGF.

Shirakata et al. (2010)	Dogs	2-wall intrabony defects	3% hydroxypropyl cellulose 1,000–4,000 cP solution prepared as a gel-like material containing 0.3% rhbFGF	1. bFGF 2. EMD 3. PDGF/β-TCP 4. Sham surgery (OFD)	8 weeks	The length of junctional epithelium migration observed in the EMD group was significantly shorter than those in the OFD, bFGF, and PDGF/b-TCP groups. The amount of connective tissue adhesion (without cementum) in the OFD group was significantly greater than that in the bFGF and PDGF/b-TCP groups. Moreover, new bone formation was more extensive in the bFGF group than in the OFD and EMD groups. There was no significant difference in new bone formation between the OFD and the EMD groups. The groups treated with EMD and PDGF/b-TCP showed significantly greater cementum formation than the OFD group. No significant differences were observed between the bFGF and the PDGF/b-TCP groups in any of the histometric parameters.
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PDGF/β-TCP platelet-derived growth factor with b-tricalcium phosphate, EMD enamel matrix derivative

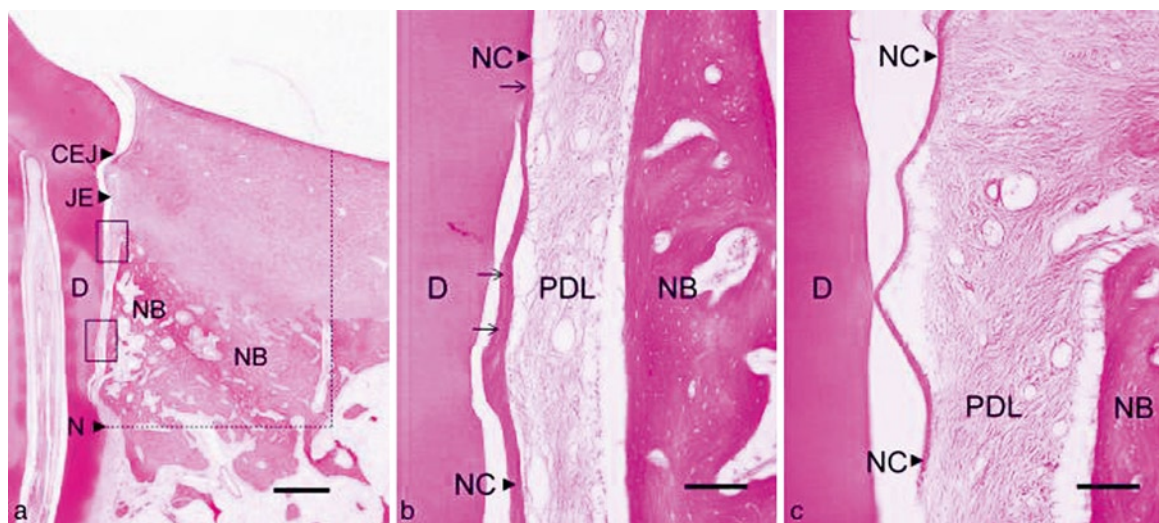


Fig. 5.18 Representative photomicrographs of a two-wall intra-bony defect treated with basic fibroblast growth factor (bFGF). (a) Overview. Note the extensive new bone (NB) formation extending from the host bone crest toward the coronal region of the defect. The dotted lines show the original defect margin (scale bar: 1 mm; haematoxylin and eosin stain). (b) Higher magnification of the apical framed area in (a). (c) Higher magnification of the coronal framed area in (a). Thick new cementum (NC) with or without collagen fibers obliquely oriented to the root surface and several blood vessels were observed (scale bar: 100 μ m; haematoxylin and eosin stain). CEJ cemento–enamel junction, JE junctional epithelium, PDL periodontal ligament, N notch (apical extent of root planing), D root dentin, arrows cementocytes (Shirakata et al. 2010. Reprinted with permission from John Wiley & Sons)

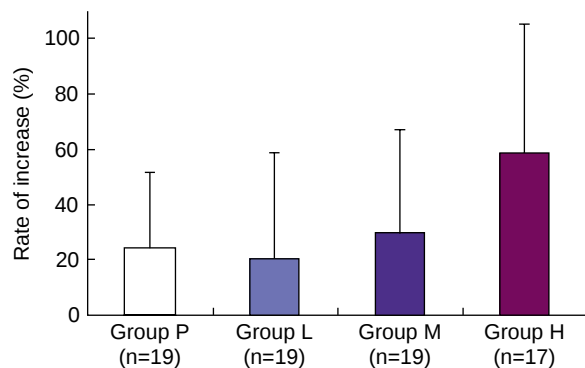


Fig. 5.19 Rates of increase in alveolar bone height in cases of 2- and 3-walled intra-bony defects. The rates of increase in alveolar bone height at 36 weeks after FGF-2 administration among Group P (19 placebo cases), Group L (19 cases administered 0.03% FGF-2), Group M (19 cases administered 0.1% FGF-2), and Group H (17 cases administered 0.3% FGF-2) were compared. This figure shows mean increase rates (%) and standard deviations of alveolar bone height. While no significant difference was observed between Groups L and M and P, Group H showed significantly increased ($P=0.021$) alveolar bone height in the bone defect region compared to Group P (Kitamura et al. 2008. PLoS ONE doi:10.1371/journal.pone.0002611.g004)

and Viridi 1999; Teare et al. 2008) (Fig. 5.20). TGF-beta1 is readily detectable in gingival crevicular fluid and increases transiently following periodontal surgery. This suggests that changes in the levels of this growth factor in gingival crevicular fluid might be useful for monitoring the progress of periodontal repair and regeneration (Kuru et al. 2004).

Recent studies have shown that enamel matrix derivative contains transforming growth factor-beta (Nagano et al. 2006; Bosshardt 2008), and is able to stimulate bone morphogenetic protein, transforming growth factor-beta and connective tissue growth factor expression in osteoblastic cells (He et al. 2004; Suzuki et al. 2005; Heng et al. 2007). The synergistic interactions between multiple biomolecules may possibly play an important regulatory role in periodontal tissue regeneration (Suzuki et al. 2005). The incorporation of platelet-rich plasma (PRP), an autologous blood product, into sinus grafts has also been proposed as a method to reduce periodontal healing times, enhance periodontal wound healing and improve bone quality, because PRP contains high concentrations of several growth factors (such as TGF- β and PDGF) and

and Viridi 1999; Teare et al. 2008) (Fig. 5.20). TGF-beta1 is readily detectable in gingival crevicular fluid and increases transiently following periodontal surgery. This suggests that changes in the levels of this growth factor in gingival crevicular fluid might be useful for monitoring the progress of periodontal repair and regeneration (Kuru et al. 2004).

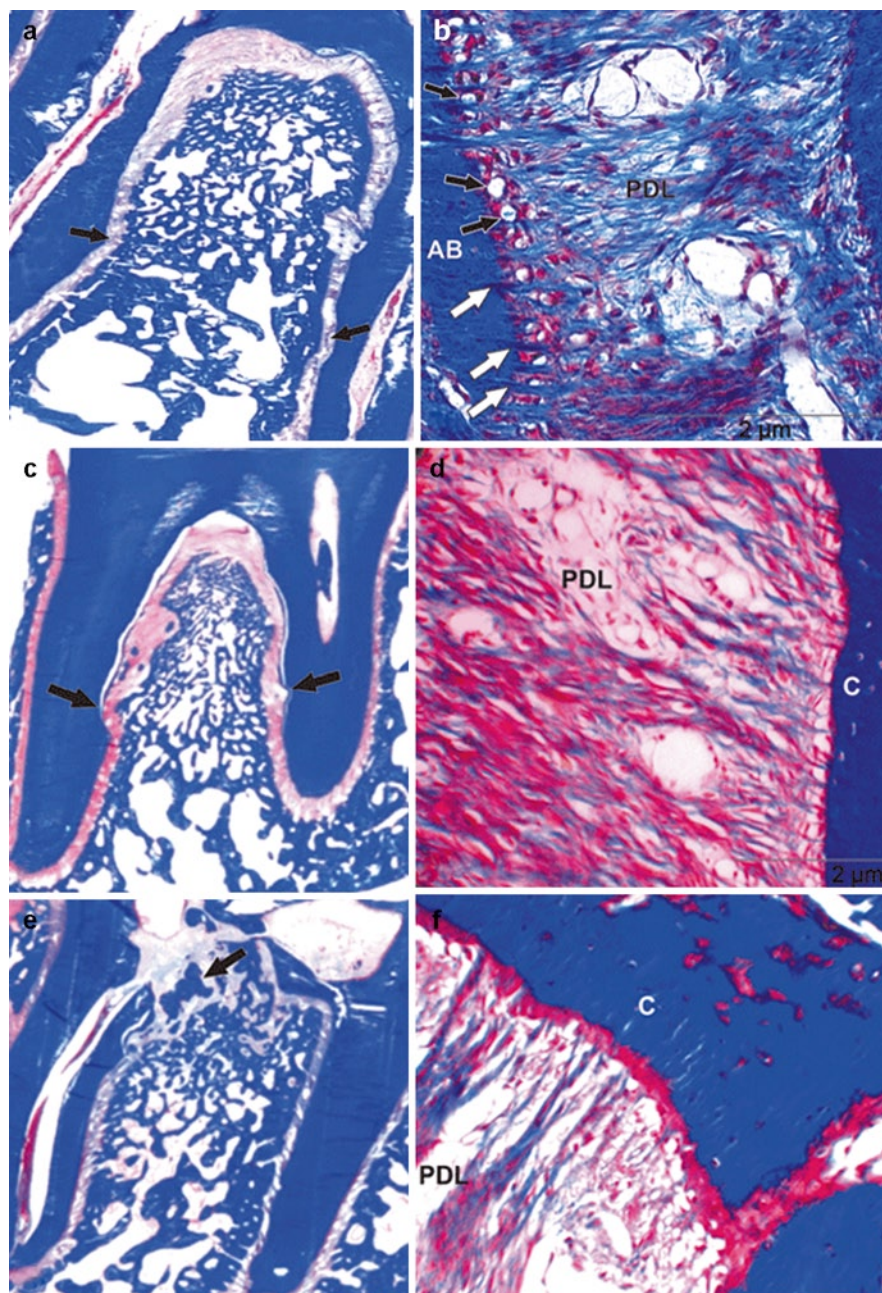


Fig. 5.20 Periodontal tissue regeneration by: (a) Recombinant human transforming growth factor- β 3 in Matrigel[®] carrier. The arrows indicate the notches made at the time of implantation. Magnification $\times 1.25$. (b) Recombinant human transforming growth factor- β 3 in Matrigel[®] carrier. New periodontal ligament with repetitive pattern of capillaries along the edge of alveolar bone (black arrows) and well-defined insertion of Sharpey's fibers (white arrows). Magnification $\times 40$. (c) Recombinant human transforming growth factor- β 3 plus muscle tissue in Matrigel[®] carrier. Arrows indicate the notches made at the time of implantation. Magnification $\times 1.25$. (d) Recombinant human

transforming growth factor- β 3 plus muscle tissue in Matrigel[®] carrier. New periodontal ligament with vascularization and insertion of Sharpey's fibers into new cellular cementum. Magnification $\times 40$. (e) Periodontal tissue regeneration by heterotopically induced bone. Magnification $\times 1.25$. The arrow indicates the osteogenic invasion of pulp cavity as a result of dentine disruption during the preparation of the defect. (f) Insertion of Sharpey's fibers into the cementoid matrix of cementum. Magnification $\times 40$. Modified Goldner's stain. AB alveolar bone, C new cellular cementum, PDL periodontal ligament (Teare et al. 2008. Reprinted with permission from John Wiley & Sons)

adhesive glycoproteins (Chen et al. 2009; Okuda et al. 2003). Platelets are enriched by 338% in the platelet-rich plasma preparation, and the concentrations of platelet-derived growth factor and transforming growth factor-beta1 in platelet-rich plasma have been reported to be 41.1 and 45.9 ng/mL, respectively. The amounts of TGF- β and PDGF present in the platelet-free plasma were minimal, indicating that almost all of the growth factor/cytokine present in the PRP gel is derived from the platelets (Landesberg et al. 2000).

Several animal studies have evaluated the TGF- β 1 and TGF- β 3 effect on periodontal regeneration. Mohammed et al. (1998) treated standardized class II furcation defects in a sheep model. The experimental design included a control group and two experimental groups: group A (80 μ m/mL TGF- β) and group B (80 μ m/mL TGF- β covered with a barrier membrane). After 6 weeks after surgery, more bone was present in group A (TGF- β only) than in the control group ($P < 0.02$) and also in group B (TGF- β + GTR) when compared with both group A and the control group ($P < 0.02$ and $P < 0.44$), respectively. This study demonstrated that TGF- β encouraged bone regeneration in class II furcation defects in sheep, an effect enhanced by the presence of a barrier membrane.

In contrast, the treatment with TGF- β 1, alone or combined with a GTR membrane, of supraalveolar periodontal defects in dogs was of limited clinical benefit, under the conditions (dose, carrier, defect type) evaluated (Wikesjö et al. 1998; Tatakis et al. 2000). The implantation into critical-size, supraalveolar periodontal defects in dogs of TGF- β 1 has demonstrated an accelerated biodegradation of a particulate calcium carbonate biomaterial, indicating a biologic activity of the TGF- β 1 formulation apparently not encompassing enhanced or accelerated periodontal regeneration (Koo et al. 2007).

The transforming growth factor- β 3 isoform is considered to be far more potent, as a regulator of functions associated with osteogenesis and angiogenesis, than transforming growth factor- β 1 or - β 2 and has significantly enhanced periodontal tissue regeneration in the nonhuman primate, *P. ursinus*, when implanted in class II furcation defects, after either direct application of the morphogen to the defects or the transplantation of recombinant human TGF- β 3-induced heterotopic ossicles. Both methods have shown a remarkable potential for the regeneration of

alveolar bone, periodontal ligament and cementum within the exposed furcations (Teare et al. 2008).

5.8 Bone Morphogenetic Proteins

The term “bone growth” can encompass many events, including developmental establishment of skeletal elements, enlargement of bone primordia and elongation of long bones. The bone may be formed via endochondral or intramembranous pathways, and may be ectopic or at bony sites. The unifying characteristic is that this bone growth occurs when precursor mesenchymal cells or osteoprogenitors are stimulated with secreted molecules that are members of the TGF- β superfamily of growth and differentiation factors (Leboy 2006).

BMP are multifunctional autocoids which have effects on many tissue types in the body and modulate embryological growth and differentiation as well as cellular function. They are members of the transformation growth factor- β (TGF- β) superfamily except for BMP-1, which does not have the C terminal sequence of the TGF- β family (Matthews 2005; Chen et al. 2004; Ducy and Karsenty 2000; Rider and Mulloy 2010). BMPs are misleadingly named, as their actions have been implicated in the development of many tissues, including heart, gut and kidney. Because BMPs have been isolated from different species, and due to the lack of standardization of these purifications, several of the BMPs have alternate names that are often used interchangeably. For instance, BMP-7 is OP-1, BMP-8 is OP-2, BMP-12 is growth and differentiation factor 7 (GDF-7), and BMP-13 is both GDF-6 and CDMP-2 (Matthews 2005). They have been classified into several subgroups according to their structural similarities (Ducy and Karsenty 2000). Jointly, this is a family of some 20 highly related cytokines within the larger TGF- β (transforming growth factor- β) superfamily.

BMPs bind to BMP receptors types I and II, both of which are required for signal transduction. There are seven distinct BMP type I receptors, including ALK-2, ALK-3 (BMPRIA) and ALK-6 (BMPRIB), and BMP type I receptor mainly determines the specificity of the intracellular signals. BMP type II also has distinct receptors, such as BMP type II receptor (BMPRII), activin type II receptor (ActR-II) and activin type IIB receptor (ActR-IIB) (Fig. 5.21).

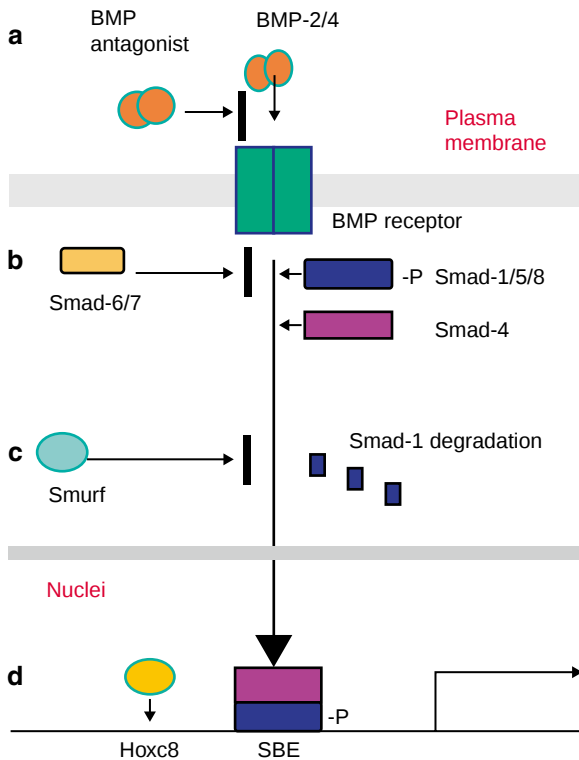


Fig. 5.21 BMP signaling and its inhibition. There are four distinct inhibitions for BMP signaling. (a) BMP antagonists inhibit binding of BMP-2/4 to their receptors. (b) Smad-6/7 completes binding to the BMP receptors with smad-1/5/8 for the inhibition of BMP signaling. (c) Smurf-2 promotes smad-1 degradation after ubiquitination. (d) Transcription factor (Hoxc8) binds to target genes to inhibit BMP-induced transcription (Abe 2006. Reprinted with permission from John Wiley & Sons)

ALK-3 is ubiquitously expressed in most types of cells including osteoblasts. Chondrocytes and osteoclasts are known to express only ALK-6. A common feature of the TGF- β superfamily has seven conserved cysteins. BMPs have two extra domains in addition to the structure identified by another TGF family, TGF- β , activin/inhibin or nodal. BMPs are translated as a large molecule with a signal peptide and cleaved to be a dimeric form of mature protein with 25 kDa after secretion. Currently, over 20 distinct BMPs have been identified and each of them has slightly different affinities to their receptors. BMP-2, -4, -6 and -7 (OP-1) are widely accepted to have osteoinductive activities *in vivo*. Other BMPs, 12 namely, GDF (growth differentiation factor)-5, -6 and -7 (BMP-12) and activin/inhibin family, exhibit low osteoinductive

activity by themselves, but modulate BMP action. Both BMP-2 and -4 preferentially bind to ALK-3 and -6 and transduce signals through smad-dependent and/or -independent mechanisms (Abe 2006). Solved high-resolution three-dimensional structures are found in the PDB for BMPs 1, 2, 3, 6, 7 and 9, and for GDF-5 (Supplementary Table S1 available at <http://www.BiochemJ.org/bj/429/bj4290001add.htm>). Besides the ability to induce bone formation, which gave the BMPs their name, the BMP/GDFs display morphogenetic activities in the development of a wide range of tissues (Rider and Mulloy 2010).

The osteogenic proteins of the TGF- β superfamily are the common molecular initiators deployed for embryonic development and the induction of bone in postnatal osteogenesis, whereby molecules exploited in embryonic development are redeployed in postnatal tissue morphogenesis as a recapitulation of embryonic development. The pleiotropy of the osteogenic proteins of the TGF- β superfamily is highlighted by the apparent redundancy of molecular signals initiating bone formation by induction including the TGF- β isoforms per se, powerful inducers of endochondral bone but in the primate only (Ripamonti 2006; Ripamonti 2007). Bone induction by the TGF- β isoforms in the primate is site and tissue specific with substantial endochondral bone induction in heterotopic sites but with absent osteoinductivity in orthotopic calvarial sites on day 30 and only limited osteogenesis pericranially on day 90 (Ripamonti and Duneas 1998; Ripamonti et al. 1997; Duneas et al. 1998). The tissue response elicited by heterotopic implantation of demineralized bone matrix is reminiscent of embryonic bone development (Fig. 5.22) (Ripamonti and Renton 2006). The strikingly pleiotropic effects of the bone morphogenetic and osteogenic proteins (BMPs/OPs) spring from amino acid sequence variations in the carboxy-terminal domain and in the transduction of distinct signaling pathways by individual Smad proteins after transmembrane serine/threonine kinase complexes of type I and II receptors. Amino-acid sequence variations amongst BMPs/OPs in the carboxy-terminal domain confer the structure/activity profile responsible for the pleiotropic activity that controls tissue induction and morphogenesis of a variety of tissues and organs by different BMPs/OPs which are helping to engineer skeletal tissue regeneration in molecular terms

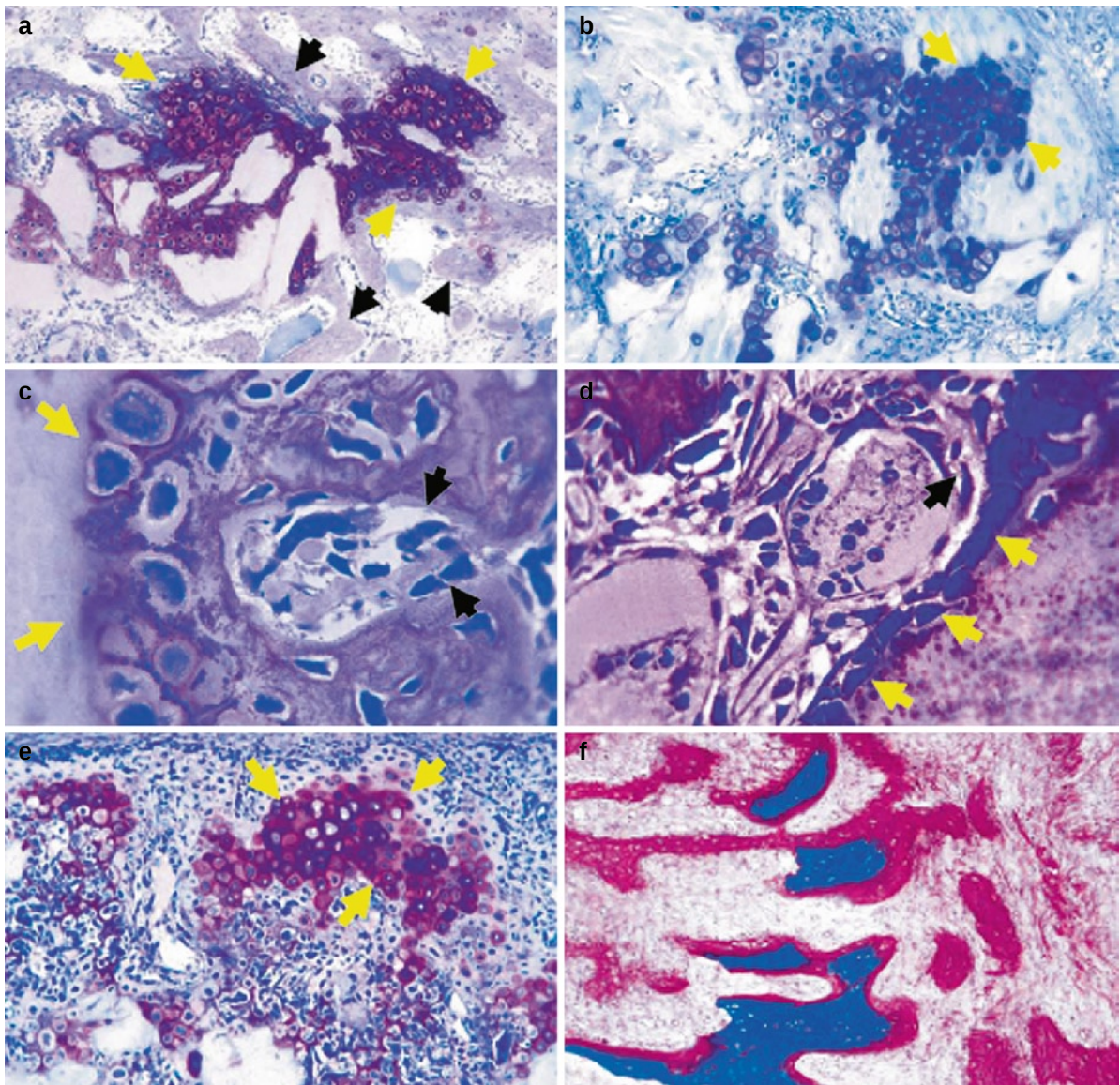


Fig. 5.22 Soluble molecular signals and the initiation of endochondral bone formation as a recapitulation of embryonic bone development. (a) and (b) Endochondral bone formation in the subcutaneous space of the rodent after implanting 5–20 μ g of naturally derived bone morphogenetic and osteogenic proteins (BMPs/OPs), purified from baboon bone matrices after gel-filtration chromatography on Sephacryl S-200. Islands of cartilage anlagen (yellow arrows) between particles of collagenous matrix, vascular invasion and bone differentiation by induction (black arrows) are shown. (c) and (d) Angiogenesis and induction of bone after implantation of 0.1–0.5 μ g doses of the final gel-eluted osteogenin fractions purified to apparent homogeneity after electroendosmotic elution. (c) Chondroblastic differentiation attached to the matrix (white arrows), vascular invasion and differentiation of osteoblast-like cells (black arrows) secreting bone matrix. (d) Capillary invasion and angiogenesis surfacing the implanted matrix as carrier with the endothelium almost touching the osteoblast-like layer attached to the matrix (white

arrows), confirming the intimate and exquisite relationships between invading capillaries and osteoblastic cell differentiation. The black arrow points to an endothelial cell migrating from the vascular compartment to the extracellular matrix compartment. (e) Induction of cartilage anlagen (white arrows) as a recapitulation of embryonic development with angiogenesis, osteoblastic cell differentiation and bone matrix deposition 11 days after subcutaneous implantation of 2.5 μ g of human recombinant osteogenic protein-1 (hOP-1). (f) Bone formation by induction in heterotopic extraskeletal sites of the nonhuman primate, *Papio ursinus*, 30 days after implantation of 100 μ g of hOP-1 per gram of gamma irradiated bovine collagenous bone matrix as carrier. Newly formed mineralized bone is shown in blue, surfaced by thick osteoid seams in orange/red. (a–f) Undecalcified sections, cut at 5 μ m, stained free-floating with Goldner's trichrome. Original magnifications: $\times 90$ (a), $\times 125$ (b), $\times 275$ (c), $\times 275$ (d), $\times 125$ (e) and $\times 90$ (f). (Ripamonti and Renton 2006. Reprinted with permission from John Wiley & Sons)

(Ripamonti 2006; Ripamonti 2007). In situ hybridization, immunolocalization, and *in vivo* studies in mammals, including human primates, have indicated that fruit flies, frogs and fractures share common morphogenetic and inductive mechanisms (i.e., the pleiotropic cascade of activities of BMP/OP gene products) (Ripamonti and Renton 2006).

The mosaicism of the expression of gene products during embryonic development is recapitulated postnatally by the exogenous application of highly purified, naturally derived, BMPs/OPs and of single recombinant osteogenic proteins (Fig. 5.23) (Ripamonti and Renton 2006).

The most promising growth factors among bone morphogenetic proteins are particularly bone morphogenetic protein-2 and bone morphogenetic protein-7, the same growth factors that are approved and applied in the orthopedic field for hard-to-heal cases (i.e. non-union, open tibial fractures and spinal fusions), but only when all other treatment options have failed (Bosshardt and Sculean 2009). The demonstrated ability of bone morphogenetic proteins to generate substantial quantities of bone suggest many applications in the oral cavity where this is the only tissue desired (Cochran and Wozney 1999). BMPs have shown potent effects for craniofacial surgery, implant site development, sinus floor elevation, segmental/resection defects and alveolar ridge augmentation (Hallman and Thor 2008; Jung et al. 2003, 2008; Samartzis et al. 2005; Garg 2010; Park 2009; Davies and Ochs 2010; Herford and Boyne 2008; Azari et al. 2002; Huang et al. 2008; Marukawa et al. 2001; Smith et al. 2008).

It has been demonstrated that BMPs/OPs regulate tooth morphogenesis at different stages of development (Fig. 5.24) (Ripamonti and Renton 2006; Ripamonti 2007; Aberg et al. 1997; Xu et al. 2009) and also the induction of cementogenesis, periodontal ligament and alveolar bone (Ripamonti 2007; Aberg et al. 1997; Thesleff and Sharpe 1997; Hogan 1996; Vainio et al. 1993; Helder et al. 1995, 1998; Thomadakis et al. 1999; Yamashiro et al. 2003; Yamamoto et al. 2004; Kémoun et al. 2007). BMPs are known to induce bone formation in ectopic sites and have been successfully used to heal critical-sized bone defects (Einhorn 2003; McKay and Sandhu 2002). In general, BMPs exert multiple effects on bone by: (1) acting as mitogens on undifferentiated mesenchymal cells and osteoblast precursors, (2) inducing the expression of the osteoblast phenotype (e.g. increasing alkaline phosphatase activity in bone

cells) and (3) acting as chemoattractants for mesenchymal cells and monocytes as well as binding to extracellular matrix type IV collagen (Trombelli and Farina 2008). In contrast to transforming growth factor- β , bone morphogenetic proteins does induce ectopic bone formation (Oates et al. 1993; Hobbs et al. 1999; Lieberman et al. 2002; Irie et al. 2003).

The requirements for structural properties of a carrier technology depend on the particular indication, inlay or onlay, and whether to use an implantable or injectable minimally invasive approach. It is essential for any carrier technology to maintain its structural integrity at the target site while releasing bone morphogenetic proteins in the desired concentrations over time. At the same time, it should resorb to not obstruct bone formation, or remain *in situ* to potentially compromise the physiological and biomechanical properties of bone (Ripamonti et al. 2001b; Huang et al. 2008; Haidar et al. 2009a, b) (Table 5.5). Many carrier materials have been investigated for the combination with naturally derived and recombinant hBMPs/OPs, as the underlying mechanism of osteogenesis in these and other bone induction studies is greatly influenced and controlled by the structural geometry of the substratum (Ripamonti et al. 2001b; Kuboki et al. 2002; Bessa et al. 2008; Haidar et al. 2009a, b). Release kinetics of BMP may have important effects on the outcome of BMP-induced periodontal regeneration. New bone formation may be affected by rapid-release kinetics. In contrast, new cementum formation is promoted by slow release of BMP (Talwar et al. 2001; King et al. 1998a; Elangovan et al. 2009). Aside from the property of influencing release kinetics, the other properties of the scaffold including elasticity, surface area, surface properties, porosity and immunogenicity, are also considered important (Engler et al. 2006; Shin et al. 2003). Furthermore, treatment of intrabony periodontal defects with BMPs are likely to not only require appropriate temporal release of the agent, but also adaptation of a carrier that is robust enough to maintain its integrity around the coronal aspect of the root in order to provide space maintenance and support the mucoperiosteal flap (King 2001). Biomimetic biomaterial matrices that sculpt bone tissue morphology should be designed so as to obtain selected biological responses by embedding the molecular signals of the BMPs/OPs superfamily within the geometric topography of the substratum. Bone tissue engineering starts by erecting scaffolds of smart biomimetic

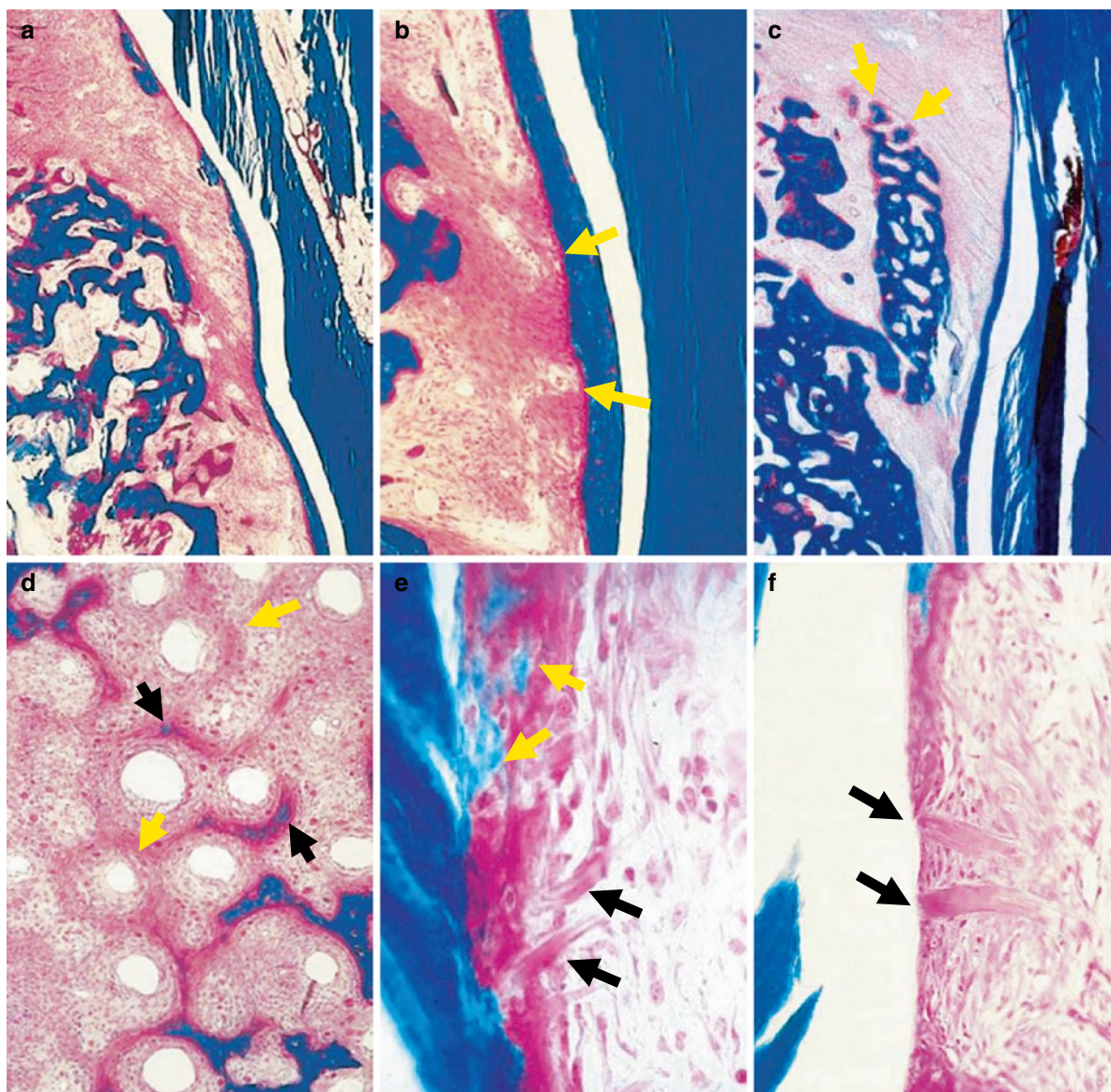


Fig. 5.23 Tissue induction, morphogenesis and regeneration as a recapitulation of embryonic development in furcation defects of mandibular molars of the nonhuman primate, *Papio ursinus*, implanted with naturally derived bone morphogenetic and osteogenic proteins (BMPs/OPs), extracted and purified from bovine bone matrices greater than 70,000-fold and predominantly containing BMP-3 (30, 33, 56). (a) Newly formed Sharpey's fibers coursing from regenerated alveolar bone in blue inserting into newly formed mineralized cementum 60 days after the implantation of 250 lg of highly purified osteogenic fractions. (b) High-power detail of the previous section, showing the assemblage of the newly formed periodontal ligament system inserting into mineralized cementum in blue (arrows). (c) Periodontal tissue regeneration in another furcation defect, treated with bovine BMP/OPs delivered by allogeneic collagenous matrix as a carrier, showing newly formed mineralized alveolar bone surfaced by osteoid seams (arrows) and Sharpey's fibers uniting the newly formed bone to the regenerated cementum. (d) In another specimen of a furcation defect treated with naturally derived

BMPs/OPs, angiogenesis and vascular invasion is observed, with formation of cellular condensations around the invading capillaries and osteoid-like tissue deposition with foci of mineralization and the induction of bone formation as a ripple-like cascade of events centered around the invading capillaries during angiogenesis as a prerequisite for osteogenesis. (e) Deposition of cemental matrix, as cementoid yet to be mineralized, on the planed dentinal surface after implantation of 250 lg of naturally derived bovine BMPs/OPs. Yellow arrows indicate foci of mineralization within the yet-to-be-mineralized cemental collagenic matrix. Black arrows indicate generating Sharpey's fibers from the newly deposited cementoid. (f) Higher magnification of the coronal area of (a) showing the generation of Sharpey's fibers (open arrows) and the mineralization of newly formed cementoid. (a–f) Undecalcified sections cut at 5 μ m and stained free-floating with Goldner's trichrome. Original magnifications: $\times 75$ (a), $\times 125$ (b), $\times 75$ (c), $\times 175$ (d), $\times 175$ (e) and $\times 175$ (f) (Ripamonti and Renton 2006. Reprinted with permission from John Wiley & Sons)

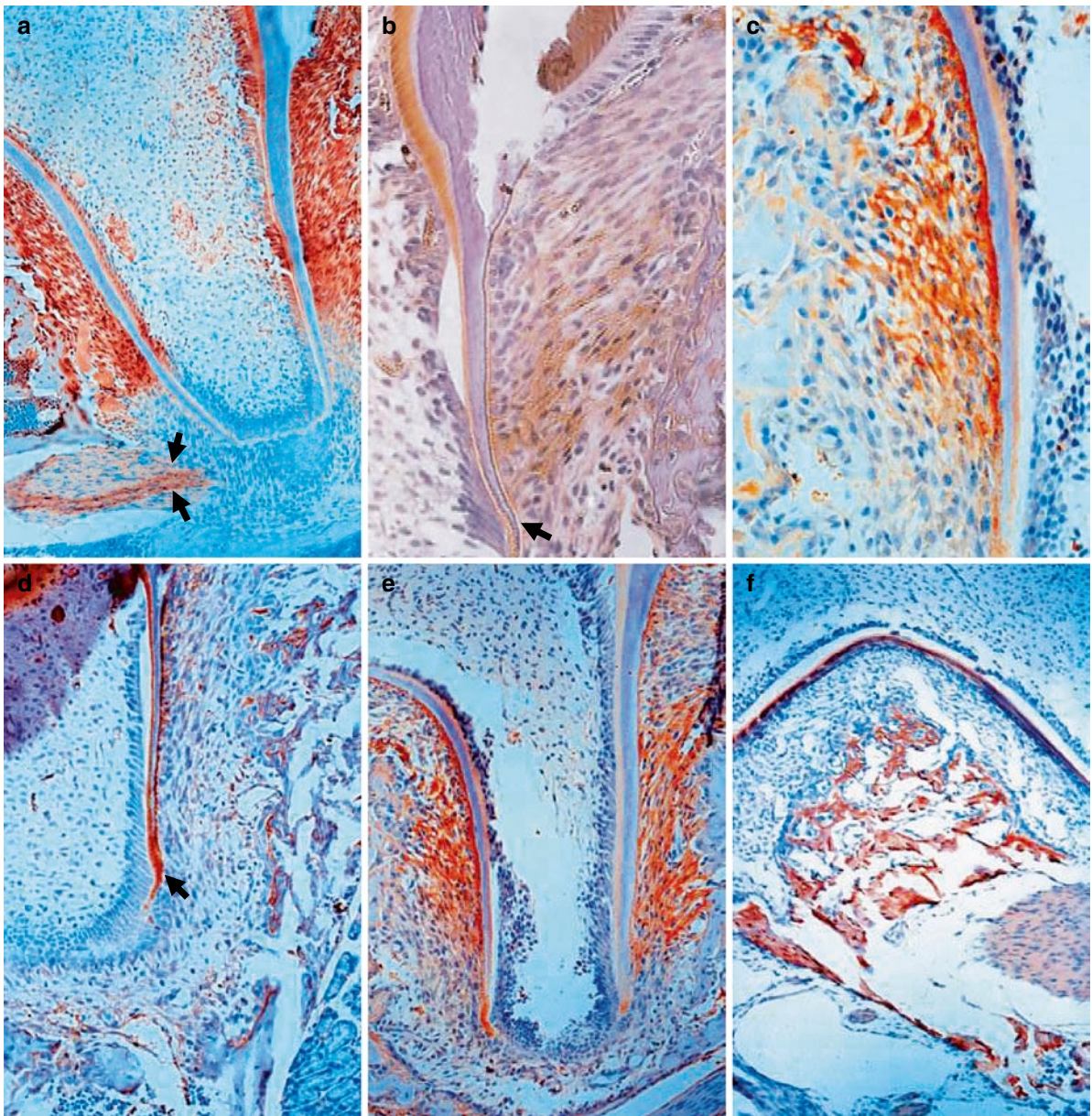


Fig. 5.24 Immunolocalization and mosaicism of expression of bone morphogenetic proteins (BMPs) during embryonic development and morphogenesis of murine periodontal tissues. **(a)** Developing mandibular molar of a 13-day-old mouse pup, showing immunolocalization of BMP-3 in the alveolar bone, periodontal ligament and cementum. Arrows indicate expression of BMP-3 in the inferior alveolar nerve. **(b)** BMP-3 expression in all three components of the periodontal tissues during tooth morphogenesis of a molar of an 11-day-old mouse pup. Note BMP-3 immunolocalization in the periodontal ligament fibers uniting the alveolar bone to the newly forming cementum (arrow). **(c)** Developing root of a mandibular molar in a 16-day-old mouse pup: strong expression of osteogenic protein-1 (OP-1) is observed during cementogenesis in cementoblasts and cementoid matrix and in developing periodontal ligament fibers.

(d) Developing root of a mandibular molar in a 16-day-old mouse pup. Strong localization of OP-1 during cementogenesis and developing fibers inserting into the newly deposited cementoid. Conspicuous staining in predentine and mantle dentine (arrow). **(e)** Developing root of a molar tooth in a 140-day-old mouse pup: OP-1 signals strongly in cementum and in the periodontal ligament space, delineating the assemblage of the periodontal ligament system together with cementogenesis. **(f)** Immunolocalization of BMP-2 in the furcation area of a 16-day-old mouse pup. BMP-2 signals in alveolar bone and predentine. Note the lack of staining in the periodontal ligament and cementum. Original magnifications: $\times 90$ (a), $\times 90$ (b), $\times 145$ (c), $\times 125$ (d), $\times 90$ (e) and $\times 75$ (f). (Ripamonti and Renton 2006. Reprinted with permission from John Wiley & Sons)

Table 5.5 General requirements for BMP delivery systems

- Biocompatibility
- Predictable biodegradability
- Low immunogenicity and antigenicity
- Enhancement of cellular vascularization and attachment
- Affinity to BMPs and bone (defect)
- Maintenance and enhancement of BMP bioactivity
- Controlled and metered/tailored protein release at an effective dose for the appropriate period of time according to defect anatomical site, size and vascularity
- Malleability and ease of manufacture
- Safety, stability, sterility, availability and cost-effectiveness
- Regulatory agencies approval for the desired clinical indication and application

Source: Haidar et al. (2009b). Reprinted with permission from Springer

matrices affecting the release of osteogenic soluble molecular signals. The molecular scaffolding lies at the heart of all new tissue engineering strategies: morphogens exploited in embryonic development can be re-exploited and redeployed for the initiation of post-natal morphogenesis and regeneration. BMPs/OPs, soluble molecular osteogenic signals of the TGF- β supergene family, are sculpting tissue constructs that engineer skeletal tissue regeneration in molecular terms (Ripamonti et al. 2005). An absorbable collagen sponge was the first bone morphogenetic protein carrier technology to be approved by the United States Food and Drug Administration (HelistatTM, Integra Life Sciences, Plainsboro, NJ, USA). The absorbable collagen sponge is a bovine type I collagen matrix that is soak-loaded with a bone morphogenetic protein solution before surgical implantation. The recombinant human bone morphogenetic protein-2/absorbable collagen sponge construct has shown clinical efficacy for a number of indications; however, it is vulnerable to tissue compression and thus appears less effective for onlay indications. The recombinant human bone morphogenetic protein-2/absorbable collagen sponge has been combined with various other biomaterials including hydroxyapatite and bioglass technologies, organic polymers including allogeneic/xenogeneic bone collagen matrices, and space-providing occlusive or porous, resorbable or nonresorbable devices to improve its structural integrity (Huang et al. 2008).

The outcomes of experiments that have investigated the efficacy of added bone morphogenetic proteins in animal and human periodontal defect models have

been exhaustively presented and discussed in a considerable number of reviews (Danesh-Meyer 2000; Giannobile and Somerman 2003; Lee et al. 2010a; Elangovan et al. 2009; Trombelli and Farina 2008; Ripamonti and Reddi 1994, 1997; Wikesjö et al. 2009; Kaigler et al. 2006; Ripamonti et al. 2005, 2009a, 2009b; Ripamonti 2006; Taba et al. 2005; Ripamonti 2007; Ripamonti and Petit 2009; Ripamonti and Renton 2006; Huang et al. 2008; Cochran and Wozney 1999; Raja et al. 2009; Kao et al. 2009; Moore et al. 2010; Chen et al. 2009, 2010).

In the field of periodontal regeneration, much of the animal studies research interest has focused on bone morphogenetic protein-2 (OP-2) (Sorensen et al. 2004; Chen et al. 2007b; Sigurdsson et al. 1995, 1996; Wikesjö et al. 1999, 2003a, c, 2004; Kinoshita et al. 1997; Blumenthal et al. 2002; Choi et al. 2002; Saito et al. 2003; Takahashi et al. 2005; Ishikawa et al. 1994; King et al. 1999; King et al. 1997, 1998a, b), bone morphogenetic protein-3 (osteogenin) (Ripamonti et al. 1994; Bowers et al. 1991), bone morphogenetic protein-6 (Huang et al. 2005), bone morphogenetic protein-7 (OP-1) (Ripamonti et al. 1996, 2002, 2001a; Giannobile et al. 1998), bone morphogenetic protein-12 (Wikesjö et al. 2004) and bone morphogenetic protein-14 (Growth/differentiation factor-5 GDF-5) (Kwon et al. 2010a, b, c; Lee et al. 2010b; Kim et al. 2009) (Fig. 5.25). The studies designs and main results are summarized in Table 5.6.

Carrier technologies have included bioresorbable poly(D,L-lactide-co-glycolide) (PLGA) microparticles, allogeneic demineralized bone matrix (DBM), an absorbable bovine type I collagen sponge (ACS), bovine bone mineral (Bio-Oss), polylactic acid granules (Drilac), a calcium phosphate cement (Ceredex) and a hyaluronan sponge (Lee et al. 2010b) (Figs. 5.26 and 5.27).

Nonresorbable (Wikesjö et al. 2003b, 2003c) and biodegradable (Wikesjö et al. 2003a) macroporous space-providing devices have been used in some studies (Lee et al. 2010a). RhBMP-2 dosages have ranged between 0.05 and 0.4 mg/mL and healing intervals have ranged between 10 days and 24 weeks (Lee et al. 2010a). Application of rhBMP-2 induced significant bone formation, depending on the carrier system and the presence or absence of space-providing devices. Also, significant formation of cementum or a cementum-like substance on the root surface was induced (Lee et al. 2010b).

Extracts from the collagenous matrix in bone include not only bone morphogenetic proteins but also other growth factors. The yield of extracted and partially purified bone morphogenetic proteins only

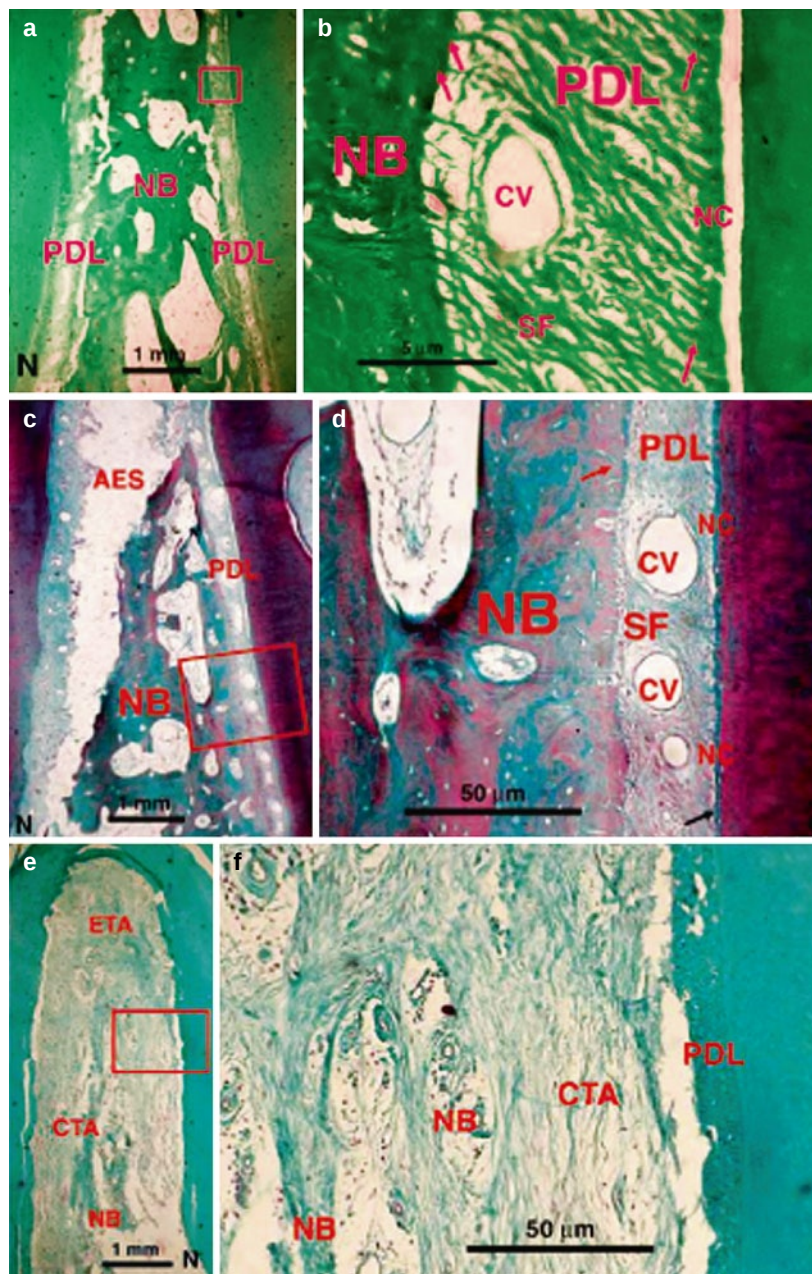


Fig. 5.25 Evaluation of the biological activity enhancement of a novel glycidyl methacrylated dextran (Dex-GMA)/gelatin hybrid hydrogel containing microspheres loaded with bone morphogenetic proteins (BMP) as periodontal cell/tissue scaffold in dogs. Histological examination of periodontal tissue regeneration (modified Mallory's trichrome staining methods, NB new bone, NC new cementum, PDL new periodontal ligaments, N notch, SF Sharpey's fibers, CV capillary vessel, AES area of empty space, CTA connective tissue area, ETA epithelium tissue area) in different groups over 8 weeks post-surgery (original magnification: (a), (c), and (e) $\times 12.5$; (b) $\times 1,000$; (d) and (f) $\times 100$). In BMP-MP/scaffold group (a) and (b), significant amounts of new bone (a) and adequate width of periodontal ligament (b) were observed. The denuded root surface was almost separated the new bone from cementum. On the denuded root surfaces of the

furcation area, newly formed cementum covered the surface (b), and Sharpey's fibers inserted into the cementum were frequently observed (b). Where (b) is the magnified view of the rectangle frame from (a). In BMP/scaffold group, all the periodontal tissues have not got full regeneration (c), but incomplete Sharpey's fibers and bone reconstruction could also be seen (d). Where (d) is the magnified view of the rectangle frame from (c). Compared with the two BMP containing groups, less PDL or hard tissue regeneration was observed in the BMP-free scaffold control (e) and (f), epithelial cells invaded into the top of the furcation (e), and almost no cementum regeneration was observed in the defect area (f). Where (f) is the magnified view of the rectangle frame from (e). Note: the arrows point to Sharpey's fibers inserted into the bone and cementum (Chen et al. 2007a. Reprinted with permission from Elsevier)

Table 5.6 Bone morphogenetic proteins investigated for periodontal healing/regeneration: selective data from animal experiments

Study	Growth factor (s)	Platform	Periodontal defect treated	Carrier	Study groups	Healing interval	Main results
Sigurdsson et al. (1995)	BMP-2	Dogs	Supraalveolar defects	Synthetic bioerodable particles and autologous blood	1. rhBMP-2 2. Control vehicle	8 weeks	rhBMP-2 in a particulate delivery system result in substantial regeneration of bone and periodontal regeneration. Height of alveolar bone regeneration amounted to 3.5 ± 0.6 and 0.8 ± 0.6 mm for rhBMP-2 and control defects. Cementum regeneration averaged 1.6 ± 0.6 and 0.4 ± 0.3 mm for rhBMP-2 and control defects, respectively ($P = 0.005$).
Sigurdsson et al. (1996)	BMP-2	Dogs	Supraalveolar defects	Deminerized bone matrix (DBM), bovine deorganified crystalline bone matrix (Bio-Oss), an absorbable collagen sponge (ACS) of type I bovine collagen, poly(D,L-lactide-co-glycolide) microparticles (PLGA), and polylactic acid granules (Drilac)	1. DBM/rhBMP-2 2. DBM 3. Bio-Oss/rhBMP-2 4. ACS/rhBMP-2 5. PLGA/rhBMP-2 6. Drilac/rhBMP-2	8 weeks	Substantial bone regeneration was observed in all defects implanted with rhBMP-2. The qualities of the carrier system, including its space-maintaining capacity can affect the ability of rhBMP-2 to regenerate both alveolar bone and periodontal attachment.

Chen et al. (2007a)	BMP-2	Dogs	Furcation defects	Dex-GMA/ gelatin hybrid hydrogel containing microspheres/ scaffold	1. Scaffold only 2. BMP-MP/ scaffold (Dex-GMA/ gelatin scaffolds containing microspheres loaded with BMP) 3. BMP/scaffold (scaffolds without microspheres but adsorbed with the same amount of BMP aqueous solution)	8 weeks	There was new cementum, along with periodontal ligament and coronal growth alveolar bone in BMP-MP/scaffold group, significant amount of new bone was observed in general specimens. The denuded root surface covered with new cementum, and regenerated PDL could be seen. The regenerative tissues completely filled furcation areas above the reference notches, the regulated Sharpey's fibers inserted into the cementum were frequently observed and all periodontal tissue including capillary vessel gained reconstruction; adequate width of PDL, which separated the new bone from the cementum, and complete alveolar bone reconstruction could be seen. While in BMP/scaffold group, partially regenerated bone and few other tissues such as regular PDL and cementum could be observed. Compared with the two BMP-containing group, scaffold control group has achieved less new bone, less new cementum and PDL regeneration, and epithelial cells and connective tissue invaded into the top of the furcation.
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(continued)

Table 5.6 (continued)

Study	Growth factor (s)	Platform	Periodontal defect treated	Carrier	Study groups	Healing interval	Main results
King et al. (1997)	BMP-2	Rats	Fenestration defects	Collagen gel solution	1. Root conditioning (37% phosphoric acid, 15 s) + rhBMP-2/ collagen gel 2. Root conditioning + collagen gel 3. Root conditioning	10 and 38 days	At 38 days, in the controls, significantly greater new cementum formation occurred apically compared with coronally ($P < 0.05$). Furthermore, although apical growth in the controls was similar to that in the BMP group at both levels, a greater than 2.5-fold increase in cementum formation was observed coronally in the BMP group compared with controls. Finally, when the coronal and apical sections were analyzed in response to BMP-2 on bone formation, similar growth was observed throughout the defect (untreated-coronal, 1.63 ± 0.45, -apical, 1.44 ± 0.63, mean + SE; collagen gel-coronal, 1.64 ± 0.33, -apical, 1.43 ± 0.35; BMP-coronal, 3.72 ± 1.15, -apical, 4.09 ± 0.79). Connective tissue attachment-including percentage of root coverage by collagen bundles (test, $18.2 \pm 2.9\%$, and collagen gel control, 17.4 ± 3.3), the number (test, 17.2 ± 2.8, and collagen gel control, 22.8 ± 3.7), and width (test, $5.7 \pm 1.3 \mu\text{m}$, and collagen gel control, $7.2 \pm 1.7 \mu\text{m}$) of collagen bundles was similar in both test and the collagen gel control groups. Resorption lacunae were significantly greater in the untreated control group ($545.2 \pm 262.8 \mu\text{m}^2$) compared with the BMP group ($22.3 \pm 19.4 \mu\text{m}^2$) ($P < 0.05$), but were not significantly different from the collagen gel control group ($221.8 \pm 112.3 \mu\text{m}^2$).

King et al. (1998b)	BMP-2	Rats	Fenestration defects	Collagen gel delivery system	1. rhBMP-2/collagen gel 2. Collagen gel only 3. Untreated Each of the three groups were further subdivided into those that received prior root acid conditioning with 35% phosphoric acid gel and those without acid conditioning	10 days	The BMP groups with and without acid conditioning developed significantly more bone over the second molar ($3.89 \pm 0.86\%$ and $7.62 \pm 0.93\%$, respectively), compared with the respective controls (collagen gel only) ($1.24 \pm 0.26\%$ and $2.77 \pm 0.52\%$) and untreated groups ($1.34 \pm 0.35\%$ and $3.69 \pm 0.37\%$) ($P < 0.05$). New cementum formation was greatest in the BMP acid conditioned group ($628.4 \pm 253.8 \mu\text{m}^2$) and lowest in the non-acid conditioned UN group ($207.6 \pm 36.4 \mu\text{m}^2$) ($P < 0.05$). Results suggest that root-conditioning agents operating at low pH administered into the periodontal wound impairs early BMP-induced osteogenesis while simultaneously promoting BMP-induced cementogenesis.
King et al. (1998a)	BMP-2	Rats	Fenestration defects	Collagen membrane (Naturan medical, UK)	1. rhBMP-2/collagen membrane 2. Collagen membrane 3. Untreated	10 days	Both carrier systems for BMP-2 significantly increased new bone formation compared with controls during the early stages of periodontal wound healing. The more slowly dissolving collagen membrane carrier system for BMP-2 produced significantly greater new cementum compared with the collagen gel carrier (used in the study King et al. 1998b), suggesting that a more prolonged exposure of BMP-2 is required to increase cementogenesis. New bone formation was significantly greater in BMP/gel compared with both BMP/membrane and controls ($P < 0.05$). However, new cementum formation was significantly greater in BMP-2/membrane ($721 \pm 166 \mu\text{m}^2$) compared with collagen membrane, collagen gel, and untreated ($P < 0.02$) ($190 \pm 44 \mu\text{m}^2$, $327 \pm 114 \mu\text{m}^2$ and $172 \pm 33 \mu\text{m}^2$, respectively) and more than 1.5 times BMP/gel ($451 \pm 158 \mu\text{m}^2$).

(continued)

Table 5.6 (continued)

Study	Growth factor (s)	Platform	Periodontal defect treated	Carrier	Study groups	Healing interval	Main results
King and Hughes (1999)	BMP-2	Rats	Fenestration defects	Collagen membrane (Naturan medical, UK)	1. rhBMP-2/collagen membrane in extracted (hypofunctional) group (BMPe) 2. rhBMP-2/collagen membrane in non-extracted (functional) group (BMPf) 3. Collagen membrane only (CONe and CONF)	10 and 35 days	At 10 days, CONe developed greater bone growth compared with CONF ($P < 0.05$), while both BMP groups developed greater bone compared with controls. However, BMPe developed more ankylosis compared with both CONe and CONF while BMPf was significantly greater than CONF only ($P < 0.05$). BMPf only developed significantly greater new cementum compared with controls. At 35 days, BMP35f developed greater bone growth compared with all other groups including BMP35e ($P < 0.05$) and unlike results at 10 days, no differences were apparent between CON35f and CON35e. Unwanted bone growth beyond the defect margin anteriorly was significantly greater in BMP35f. Results suggest hypofunction stimulates early bone formation. Furthermore, hypofunction and BMP-2 increase the development of transient ankylosis. However, after wound healing is complete, function augments the early effects of BMP-2-induced new bone growth indicating remodeling to physiological levels does not occur. Finally, occlusal loading is both an important stimulus for remodeling and establishment of the periodontal ligament space during early wound healing as well as enhancing BMP-2-induced cementogenesis.

Sorensen et al. (2004)	BMP-2	Dogs	Supraalveolar defects	Calcium phosphate cement (Ceredex™, ETEX Corporation)	1. rhBMP-2/Ceredex 2. Ceredex only	12 weeks	Induced bone height in sites receiving rhBMP-2/Ceredex was twofold greater than that observed in the Ceredex control approximating 82% and 84% (rhBMP-2 at 0.2 and 0.4 mg/mL, respectively) versus 41% of the defect height. The corresponding mean values for induced bone area were 11.4 ± 4.4 and $16.2 \pm 10.1 \text{ mm}^2$ versus $1.9 \pm 1.3 \text{ mm}^2$. Bone density values appeared somewhat smaller for sites receiving rhBMP-2/Ceredex ($31.1 \pm 4.3\%$ and $26.5 \pm 3.9\%$; rhBMP-2 at 0.2 and 0.4 mg/mL, respectively) compared with that of the Ceredex control ($40.9 \pm 7.2\%$). Cementum regeneration encompassed 60% and 35% of the defect height in sites receiving rhBMP-2/Ceredex (rhBMP-2 at 0.2 and 0.4 mg/mL, respectively) compared with 27% for the Ceredex control.
Selvig et al. (2002)	BMP-2	Dogs	Supraalveolar defects	Absorbable collagen sponge (ACS)	1. rhBMP-2/ACS 4 weeks 2. rhBMP-2/ACS 8 weeks	4- and 8-week	rhBMP-2/ACS elicits a rapid osteoinductive process throughout the implant as well as along and onto the instrumented adjacent root surface. Lamellated trabecular bone was the predominant regenerated tissue. A typical cementum-periodontal ligament-alveolar bone relationship was a rare observation. Ankylosis was a frequent observation, although areas showing characteristics of a periodontal ligament with a fine layer of acellular fiber cementum and occasional inserting Sharpey's fibers were also observed.
Talwar et al. (2001)	BMP-2	Rats	Periodontal fenestration defects	Gelatin carrier cross-linked for 2 h (fast release/degradation) and 16 h (slow/degradation).	1. Control: slow degrading gelatin (CONs) 2. Control: fast degrading gelatin (CONF) 3. rhBMP-2/slow degrading gelatin (BMPs) 4. rhBMP-2/fast degrading gelatin (BMPf)	10 days	The amount of new bone formation in the BMPf group ($1.67 \pm 0.65 \times 10^{-4} \text{ m}^2$) significantly greater compared with the CONF group ($0.34 \pm 0.11 \times 10^{-4} \text{ m}^2$) ($P < 0.05$). The rhBMP-2 treated defects induced greater cementum formation than the controls. New cementum formation was significantly greater in the BMPs group ($617.60 \pm 147.00 \text{ } \mu\text{m}^2$) compared with the CONs, CONF, and BMPf groups ($233.54 \pm 108.03 \text{ } \mu\text{m}^2$, $75.69 \pm 41.83 \text{ } \mu\text{m}^2$, $265.94 \pm 89.66 \text{ } \mu\text{m}^2$, respectively) ($P < 0.05$).

(continued)

Table 5.6 (continued)

Study	Growth factor (s)	Platform	Periodontal defect treated	Carrier	Study groups	Healing interval	Main results
Wikeshj� et al. (1999)	BMP-2	Dogs	Supraalveolar defects	Absorbable collagen sponge (ACS)	1. rhBMP-2/ACS (rhBMP-2 at 0.05 mg/mL) 2. rhBMP-2/ACS (rhBMP-2 at 0.10 mg/mL) 3. rhBMP-2/ACS (rhBMP-2 at 0.20 mg/mL) 4. Buffer/ACS	8 weeks	Supraalveolar periodontal defects receiving BMP-2 at 0.05, 0.10 or 0.20 mg/mL exhibited extensive alveolar regeneration comprising 86%, 96%, and 88% of the defect height, respectively. Cementum regeneration encompassed 8%, 6% and 8% of the defect height, respectively. Ankylosis was observed in almost all teeth receiving BMP-2. Root resorption and ankylosis appeared correlated to BMP-2 concentration presenting more frequently and in greater extents in defects receiving rhBMP-2 at 0.2 mg/mL. Supra-alveolar periodontal defects receiving BMP-2 at 0.05, 0.10 or 0.20 mg/mL exhibited root resorption of 0.2 ± 0.2 mm, 0.4 ± 0.4 mm and 0.5 ± 0.2 mm and ankylosis 0.7 ± 0.3 mm, 1.2 ± 1.3 mm and 1.3 ± 0.5 mm.
Wikeshj� et al. (2003a)	BMP-2	Dogs	Supraalveolar defects	Absorbable collagen sponge (ACS)	1. 1.rhBMP-2/ACS/ePTFE2. Buffer/ACS/ePTFE	8 weeks	rhBMP-2/ACS/ePTFE significantly enhanced bone formation compared to the control (bone height: 4.8 ± 0.3 versus 2.0 ± 0.2 mm; $P = 0.001$; bone area 10.9 ± 1.3 versus 1.4 ± 0.1 mm ² ; $P = 0.01$). The density of newly formed bone was significantly lower in sites receiving rhBMP-2/ACS/ePTFE compared to control ($28.6 \pm 5.5\%$ versus $54.5 \pm 9.0\%$; $P = 0.01$). No differences between groups were found for cementum regeneration and root resorption.

Wikesjö et al. (2003b)	BMP-2	Dogs	Supraalveolar defects	Absorbable collagen sponge (ACS)	1. rhBMP-2/ACS 2. rhBMP-2/ ACS + macropo- rous ePTFE device, stabilized with stainless- steel tacks	8 weeks	Jaw quadrants receiving rhBMP-2/ACS and the macroporous ePTFE device exhibited a mean defect height of 4.8 ± 0.3 mm which was equal to the device height, suggesting that the devices had not collapsed onto the root surface. New bone formation approximated 98%, cementum formation 44%, and ankylosis 31% of the defect height. Root resorption was limited. The wound area averaged 10.2 ± 2.2 mm ² and the bone area averaged 9.3 ± 2.7 mm ² , suggesting that the area provided by the device was almost completely occupied by newly formed bone. The bone density averaged $29.0 \pm 6.9\%$. Jaw quadrants receiving rhBMP-2/ACS without the macroporous ePTFE device exhibited a mean defect height of 4.8 ± 0.2 mm. Bone and cementum regeneration amounted to 94% and 35% of the defect height, respectively. Ankylosis approximated 46% of the defect height. Root resorption was limited. The bone area averaged 5.1 ± 1.1 mm ² . The bone density averaged $40.7 \pm 9.0\%$.
Wikesjö et al. (2003c)	BMP-2	Dogs	Supraalveolar defects	Bioresorbable hyaluronan carrier (Hy)	1. PGA-TMC membrane/ rhBMP-2/Hy 2. PGA-TMC membrane/Hy	8 weeks	Newly formed alveolar bone approached and became incorporated into the macroporous PGA-TMC membrane in sites receiving rhBMP-2. Regeneration of alveolar bone height was significantly increased in sites receiving the PGA-TMC/rhBMP 2 combination compared to control (3.8 ± 1.3 versus 0.7 ± 0.5 mm at 8 weeks and 4.6 ± 0.8 versus 2.1 ± 0.4 mm at 24 weeks; $P < 0.05$). Limited cementum regeneration was observed for PGA-TMC/rhBMP-2 and PGA-TMC control sites. Ankylosis compromised regeneration in sites receiving PGA-TMC/rhBMP-2.

(continued)

Table 5.6 (continued)

Study	Growth factor (s)	Platform	Periodontal defect treated	Carrier	Study groups	Healing interval	Main results
Wikström et al. (2004)	BMP-2; BMP-12	Dogs	Supraalveolar periodontal defects	Absorbable collagen sponge (ACS)	1. rhBMP-12 (0.04 mg/mL)/ACS 2. rhBMP-12 (0.2 mg/mL)/ACS 3. rhBMP-12 (1.0 mg/mL)/ACS 4. rhBMP-2 (0.2 mg/mL)/ACS	8 weeks	Bone regeneration appeared increased in sites receiving BMP-2/ACS compared to sites receiving BMP-12/ACS. Bone formation ranged from 0% to 20% of the defect height at sites receiving BMP-12 at 0.04 mg/mL, 0–40% of the defect height at sites receiving BMP-12 at 0.2 mg/mL, and approximated 10–30% of the defect height at sites implanted with BMP-12 at 1.0 mg/mL. Bone regeneration averaged 52%, 56% and 58% of the defect height for sites receiving BMP-12/ACS (BMP-12 at 0.04, 0.2, and 1.0 mg/mL, respectively). The corresponding value for sites receiving BMP-2/ACS approximated 71%. Bone regeneration area was similar among sites receiving BMP-12/ACS ranging from 2.1 ± 1.1 to 3.2 ± 2.1 mm ² . Cementum regeneration was similar comparing sites implanted with BMP-2/ACS to sites implanted with BMP-12/ACS. Continuous cementum regeneration averaged 1.4 ± 0.8 mm (24% of the defect height) for sites receiving BMP-12 (1.0 mg/mL)/ACS. The corresponding values for sites receiving BMP-12 (0.04 mg/mL)/ACS, BMP-12 (0.2 mg/mL)/ACS or BMP-2/ACS were 2.2 ± 1.0 (37%), 2.4 ± 1.3 (41%), and 2.5 ± 1.4 mm (43%), respectively. In contrast, sites receiving BMP-12/ACS exhibited a functionally oriented PDL bridging the gap between newly formed bone and cementum whereas this was a rare observation in sites receiving BMP-2/ACS. Ankylosis appeared increased in sites receiving BMP-2/ACS compared to those receiving BMP-12/ACS
Kinoshita et al. (1997)	BMP-2	Dogs	Horizontal circumferential defects	Sponge-type carrier material made of gelatin and polylactic acid polyglycolic acid copolymer	1. rhBMP-2/carrier 2. Carrier only	12 weeks	Considerable new bone formation was observed in the BMP-2-treated sites. New cementum with Sharpey's fibers was observed on the instrumented root surface. On histometric analysis, the amount of new bone, new cementum, and connective tissue attachment was significantly greater in the BMP-2-treated group ($P < 0.01$).

Blumenthal et al. (2002)	BMP-2	Baboon	Three-wall intrabony periodontal defects	Collagen sponge (ACS) or a calcium phosphate putty (α BSM) (ETEX, Cambridge)	1. rhBMP-2/ACS 2. rhBMP/αBSM 3. Buffer/ACS 4. Buffer/αBSM 5. Sham-operated surgical controls	4 months	Defect sites receiving BMP-2/ACS and BMP-2/αBSM demonstrated significantly greater regeneration than controls. No significant differences were observed between defect sites receiving BMP-2/ACS or BMP-2/αBSM regarding epithelial migration and connective tissue attachment and new bone formation. There were significant differences in alveolar bone formation between ACS or αBSM versus sham controls, but not between each implant carrier alone. However, there were significant differences when rhBMP-2 were added to the both carriers (2.0 ± 0.77 versus 1.19 ± 0.26 for BMP-2/ACS versus ACS, and 1.95 ± 0.37 and 1.03 ± 0.24 for BMP-2/αBSM versus αBSM). Mean new cementum formation ranged from 0.83 ± 0.36 to 2.32 ± 0.3 mm. However, rhBMP-2/ACS supported significantly greater new cementum formation.
Choi et al. (2002)	BMP-2	Dogs	Horizontal circumferential defects	Absorbable collagen sponge (ACS)	1. rhBMP/ACS 2. Buffer/ACS 3. Sham-operated controls	8- and 24-week	Surgical implantation of BMP-2/ACS resulted in accelerated enhanced bone formation in the 3-wall intrabony periodontal defects but in no apparent enhancement of cementum regeneration. Alveolar bone regeneration averaged 3.6 ± 0.6, 2.6 ± 0.6 and 2.3 ± 0.4 mm for rhBMP/ACS, the buffer control and the surgical control at 8 weeks, respectively. At 24 weeks, the corresponding values were 3.4 ± 0.6, 2.6 ± 0.6 and 2.1 ± 0.6 mm. At 8 weeks, new bone formation amounted to $44 \pm 10\%$, $36 \pm 17\%$ and $28 \pm 17\%$ for rhBMP/ACS, the buffer control and the surgical control. At 24 weeks the corresponding values were $42 \pm 7\%$, $27 \pm 20\%$ and $17 \pm 14\%$. No sites exhibited evidence of ankylosis.

(continued)

Table 5.6 (continued)

Study	Growth factor (s)	Platform	Periodontal defect treated	Carrier	Study groups	Healing interval	Main results
Saito et al. (2009)	BMP-2	Dogs	Horizontal circumferential defects	PGS bioabsorbable carrier composed of polylactate-oligylcolate copolymer and gelatin sponge (PGS)	1. rhBMP-2/PGS 2. Physiologic saline/PGS	12 weeks	Downgrowth of junctional epithelium was significantly less in the BMP-treated group compared to the control group (1.07 ± 0.68 vs. 2.15 ± 0.45 , $P < 0.01$). The height of regenerated bone and the area of regenerated bone were significantly greater in the BMP-treated group compared to the control group (1.40 ± 0.95 vs. -0.15 ± 0.24 , respectively 0.81 ± 1.50 vs. 0.00 ± 0.00 , $P < 0.01$). Root resorption was significantly less in the BMP-treated group compared to the control group (0.39 ± 0.32 vs. 1.13 ± 0.60 , $P < 0.01$), and there were no significant differences in the width of residual bone and ankylosis between the BMP-treated group and the control group. Significantly more cementum regeneration was observed in the BMP-treated group compared to the control group (2.35 ± 0.91 vs. 0.21 ± 0.31 , $P < 0.01$).
Saito et al. (2003)	BMP-2	Dogs	Circumferential periodontal defects	PGS bioabsorbable carrier composed of polylactate-polyglycolate copolymer and gelatin sponge	1. rhBMP-2/PGS 2. rhBMP-2/PGS with a spacer membrane 3. Physiologic saline (PS)/PGS. One quadrant was left untreated	12 weeks	Both groups treated with rhBMP-2/PGS demonstrated enhanced new bone formation and connective tissue attachment with cementum regeneration when compared to the control group. Sites treated with rhBMP-2/PGS showed a greater degree of bone formation than sites treated with rhBMP-2/PGS and spacer membrane, although the latter sites showed no ankylosis.
Takahashi et al. (2005)	BMP-2	Cats	Class III furcation defects	Combination of polylactic acid-polyglycolic copolymer and gelatin sponge (PGS)	1. rhBMP-2/PGS2. PGS only	3, 6 or 12 weeks	New bone height at 3, 6 and 12 weeks after surgery were 0.70 ± 0.13 , 1.20 ± 0.13 and 1.74 ± 0.31 mm, respectively, in the PGS alone group and 1.69 ± 0.35 mm, 2.40 ± 0.26 and 2.74 ± 0.29 mm, respectively, in the BMP-2/PGS group. New bone height was significantly greater in the sites to which BMP-2 had been applied than in the control sites at any of the postoperative observation times ($P < 0.05$). Linear lengths of ankylosis at 3 and 6 weeks after surgery were 0.22 ± 0.15 mm and 0.41 ± 0.23 mm, respectively. Residual PGS was evident between the bone and root surface in the rhBMP-2/PGS group without ankylosis at 3 weeks.

Yan et al. (2010)	BMP-2; β -NGF; BMP-2 + β -NGF	Dogs	Class III furcation defects	Alginate hydrogels	1. 0.4% rhBMP-2 2. 2% β-NGF 3. 0.4% rhBMP-2 + 2% β-NGF 4. 0.2% rhBMP-2 + 1% β-NGF 5. Control group A: untreated 6. Control group B: carrier alone	8 weeks	New bone formation was more evident in the four experimental groups, but complete periodontal regeneration was not detected in any section. Histomorphometric analysis revealed that 0.4% BMP-2 + 2% β-NGF promoted the highest percentage of periodontal regeneration among all groups (57.9 ± 28.7 vs. 40.2 ± 18.4 for 0.4% BMP-2; 29.1 ± 23.2 2% for β-NGF; 48.8 ± 24.0 for 0.2% BMP-2 + 1% β-NGF). In control groups limited bone regeneration was observed. Most of the dentinal walls of the healed regions in experimental groups were covered by a thin new cementum layer. The new cellular cementum was in direct contact with root dentin, the surface of which occasionally showed the presence of resorption lacunae. The specimens of control groups also demonstrated some cementum but limited to the apical part of the defect.
Miyaji et al. (2010)	BMP-2	Dogs	Periodontal dehiscence type defects	Solution (phosphate- buffered saline) (PBS)	1. EDTA (pH 7.0) for 3 min + 0.4 mg/mL rhBMP-2 2. EDTA (pH 7.0) for 3 min + 1.0 mg/mL rhBMP-2 3. EDTA (pH 7.0) for 3 min + PBS	16 weeks	The length of newly formed cementum-like tissue (mm) was 0.17 ± 0.12 and 0.68 ± 0.61 in the surfaces conditioned with BMP-2 at 0.4 and 1.0 mg/mL, respectively. Cementum-like tissue formation in the surfaces conditioned with 1.0 mg/mL BMP-2 was significantly greater than those in the control surfaces and in the surfaces conditioned with 0.4 mg/mL BMP-2. In the control roots, no cementum-like deposit was observed. There was a BMP dose-dependent increase in the amount of cementum-like tissue. Cementum-like tissue volume in the 1.0 mg/mL BMP-2 group extended to approximately 40%, and significant differences were seen between the control and 0.4 mg/mL BMP-2 groups. The length of junctional epithelium (mm) was 1.43 ± 0.43, 0.91 ± 0.26 and 0.70 ± 0.38 in the control, 0.4 and 1.0 mg/mL BMP-2 groups, respectively. Downgrowth of the junctional epithelium in the 1.0 mg/mL BMP-2 group was significantly less than that in the control group.

(continued)

Table 5.6 (continued)

Study	Growth factor (s)	Platform	Periodontal defect treated	Carrier	Study groups	Healing interval	Main results
Huang et al. (2005)	BMP-6	Rats	Fenestration defect	Absorbable collagen sponge (Collacote, Zimmer Dental Inc., Carlsbad, CA, USA) (ACS)	1. ACS alone 2. ACS + BMP-6 (1 µg) 3. ACS + BMP-6 (3 µg) 4. ACS + BMP-6 (10 µg)	28 days	An increase in new bone and cementum formation was noted after applying a synthetic BMP-6 polypeptide to a periodontal fenestration defect in rats. Both examining factors (BMP dosages and examining levels) had significant effects on the new bone area and thickness ($P < 0.001$). Among the three BMP groups, the 3 µg group had the most pronounced effect (on both bone area and thickness) when compared with the 1 or 10 µg groups at all examined levels ($P < 0.01$), except for the new bone area at the coronal level. New cementum thickness was increased in all three BMP groups when compared with the 0 µg group, regardless of the coronal, middle and apical levels (the statistical significance was noticed between the BMP and 0 µg groups at the all levels, except 10 µg at the middle and apical levels). Again, significantly increased thickness was also found in the 3 µg group when compared with the 1 or 10 µg groups at all three levels.
Ripamonti et al. (1994)	Bovine BMP (BMP-3 and BMP-2)	Baboon (<i>Papio ursinus</i>)	Class II furcation defects	Insoluble collagenous bone matrix (ICBM)	1. Bovine BMP/ICBM 2. ICBM	60 days	Histologic analysis revealed that native BMPs in conjunction with collagenous matrix induced cementum, periodontal ligament and alveolar bone regeneration. Mineralized bone and osteoid volume were significantly greater in the BMP-treated specimens when compared to furcation defects implanted with collagenous matrix without BMPs (24.8 ± 2.2 vs. 11.8 ± 2.6 , respectively 1.98 ± 0.19 vs. 0.98 ± 0.23). The reduction of bone (mineralized bone + osteoid) volumes for the two treatments were also statistically significant (22.8 ± 2.2 vs. 10.8 ± 2.4).

Ripamonti et al. (1996)	BMP-7 (OP-1)	Baboon	Class II furcation defects	bICBM: bovine insoluble collagenous matrix carrier	1. hOP-1 100 µg 2. hOP-1 500 µg 3. bICBM solo	60 days	Both histological and histometric analyses point to the striking induction of cementogenesis by both doses of hOP-1 with the collagenous carrier. Deposition of organized and highly cellular mineralized tissue (interpreted as cementum) had occurred on the exposed roots, often extending to the forx of the treated furcations. Control specimens showed limited regeneration, scattered remnants of the collagenous carrier, and apical migration of the junctional epithelium. Within the central regions of the exposed furcations, substantial amounts of remaining collagenous carrier were enclosed by a mineralized matrix that extended and blended into the newly deposited cementum along the root surfaces. This newly formed mineralized tissue in direct apposition to the collagenous carrier showed limited vascular invasion, and an almost complete absence of capillaries in instances of substantial matrix deposition and mineralization in close proximity to the cementum deposited on the exposed dentine. The regeneration of periodontal ligament and the insertion of functionally orientated Sharpey's fibers into cementum were also observed.
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Table 5.6 (continued)

Study	Growth factor (s)	Platform	Periodontal defect treated	Carrier	Study groups	Healing interval	Main results
Ripamonti et al. (2002)	BMP-7 (OP-1)	Baboon	Class II furcation defects	Xenogenic bovine insoluble collagenous bone matrix (ICBM)	1. ICBM alone 2. ICBM/OP-1 (0.5 mg per 1 g of carrier) 3. ICBM/OP-1 (2.5 mg per 1 g of carrier)	6 months	Furcation defects treated either with 0.5 or 2.5 mg hOP-1 per g of matrix showed the induction of alveolar bone, periodontal ligament and cementum regeneration. Induction of cementogenesis was extensive along the exposed root surfaces and also along the fornix of the furcation defects. The OP-1 also induced the generation of a functionally orientated periodontal ligament uniting the regenerated bone to the newly formed cementum. In general, although not statistically significant, the extent of new attachment and alveolar bone regeneration were greater in furcation defects treated with 0.5 mg hOP-1 than with the higher dose ($P < 0.05$). The coronal extension of new attachment formation and of alveolar bone regeneration were significantly greater in hOP-1-treated specimens than in controls ($P < 0.01$). The regenerated areas of hOP-1-treated furcation defects were significantly greater than in controls. In the latter, new attachment and alveolar bone regeneration were limited and significantly reduced compared to hOP-1-treated specimens.
Giannobile et al. (1998)	BMP-7 (OP-1)	Dogs	Class III furcation defects	Collagen vehicle	1. Surgery alone 2. Collagen vehicle 3. One of three ascending concentrations of OP-1 in a collagen vehicle (0.75 mg OP-1/g collagen, 2.5, or 7.5 mg/g)	8 weeks	Histomorphometry revealed limited evidence of osteogenesis, cementogenesis, and new attachment formation in either vehicle or surgery-alone sites. In contrast, sites treated with all 3 concentrations of OP-1 showed pronounced stimulation of osteogenesis, regenerative cementum, and new attachment formation. Lesions treated with 7.5 mg/g of OP-1 in collagen regenerated 3.9 ± 1.7 mm and 6.1 ± 3.4 mm ² of linear bone height and bone area, respectively.

Ripamonti et al. (2001a)	BMP-2 BMP-7 (OP-1)	Baboons	Class II furcation defects	Xenogeneic bovine insoluble collagenous bone matrix as	1. rhBMP-2 100 mg 2. rh OP-1 3. Combined rhOP-1/rhBMP-2	60 days	Implantation of approximately BMP-7 resulted in a twofold increase in regenerated tissue area when compared with BMP-2 alone. Combined morphogen applications resulted in less regenerated area than BMP-7 applied singly. BMP-2 applied singly to furcation defects induced greater amounts of bone, as measured histomorphometrically, than BMP-7 alone or combined morphogen applications ($P < 0.05$). Osteoid volume increased 1.5-fold in BMP-7-treated specimens over and above the values of defects treated with BMP-2 or BMP-2/BMP-7. No significant differences could be detected between treatment modalities with regard to the extent of furcation exposure. The coronal extension of new cementum formation on the distal aspect of the exposed roots was greater in BMP-7 treated specimens when compared to furcation defects implanted with BMP-2 ($P < 0.05$). hBMP-2 treated defects, on the other hand, showed limited cementum formation but a temporal enhancement of alveolar bone regeneration and remodeling. Combined applications of BMP-7 and BMP-2 did not enhance alveolar bone regeneration or new attachment formation when compared to single applications of the recombinant morphogens.
Lee et al. (2010b)	BMP-14 (GDF-5)	Dogs	Intrabony periodontal defects	β -TCP	1. rhGDF-5/ β -TCP 2. β -TCP solo 3. Sham-surgery controls	8 weeks	Cementum formation averaged ($\pm$ SD) 3.83 ± 0.73 vs. 1.65 ± 0.82 ($P < 0.05$) and 2.48 ± 1.28 mm for the controls, respectively. Corresponding values for bone regeneration height averaged 3.26 ± 0.30 vs. 1.70 ± 0.66 and 1.68 ± 0.49 mm ($P < 0.05$), and bone regeneration area 10.45 ± 2.26 vs. 6.31 ± 2.41 and 3.00 ± 1.97 mm ² ($P < 0.05$). Cementum formation included cellular/acellular cementum with or without a functionally oriented periodontal ligament. A nonspecific connective tissue attachment was evident in the sham-surgery control. Sites receiving rhGDF-5/ β -TCP or β -TCP showed some residual biomaterial apparently undergoing resorption.

(continued)

Table 5.6 (continued)

Study	Growth factor (s)	Platform	Periodontal defect treated	Carrier	Study groups	Healing interval	Main results
Kim et al. (2009)	BMP-14 (GDF-5)	Dogs	Intrabony periodontal defects	Absorbable collagen sponge	1. rhGDF-5/ACS (1 µg/defect) 2. rhGDF-5/ACS (20 µg/defect) 3. rhGDF-5/ACS (100 µg/defect) 4. Buffer/ACS	8 weeks	Surgical implantation of rhGDF-5 stimulated significant periodontal regeneration. Cementum formation was significantly enhanced in sites implanted with rhGDF-5 (1 and 100 µg) compared with control ($P < 0.05$). Similarly, bone formation height was significantly greater in sites receiving rhGDF-5 (1 and 100 µg) compared with control ($P < 0.05$). There were no significant or remarkable differences in bone and cementum formation within the selected dose interval (1, 20 and 100 µg rhGDF-5). None of the control or the rhGDF-5 sites exhibited root resorption, ankylosis or other aberrant tissue reactions.
Kwon et al. (2010c)	BMP-14 (GDF-5); PDGF	Dogs	Intrabony periodontal defects	β-TCP	1. rhGDF-5/β-TCP 2. rhPDGF/β-TCP	8 weeks	Defect height averaged ($\pm$ SD) 5.16 ± 0.43 versus 5.15 ± 0.17 mm for the rhGDF-5/β-TCP and rhPDGF/β-TCP groups, respectively ($P < 0.05$). Cementum regeneration averaged 4.49 ± 0.48 versus 2.72 ± 0.91 mm for the rhGDF-5/β-TCP and rhPDGF/β-TCP groups, respectively ($P < 0.001$). The corresponding values for bone regeneration height and area were 3.08 ± 0.74 mm and 6.03 ± 1.18 mm ² versus 1.29 ± 0.78 mm and 2.98 ± 2.61 mm ² ($P < 0.001$ and < 0.01 , respectively). The epithelial attachment averaged 0.52 ± 0.40 versus 1.17 ± 0.52 mm for rhGDF-5/β-TCP and rhPDGF/β-TCP groups, respectively ($P < 0.04$). The corresponding values for the connective tissue attachment was 0.14 ± 0.25 and 1.25 ± 0.84 mm ($P < 0.001$).
Kwon et al. (2010b)	BMP-14 (GDF-5)	Dogs	Supra-alveolar periodontal defects	β-TCP/PLGA	1. rhGDF-5/β-TCP/PLGA 2. β-TCP/PLGA (controls)	8 weeks	The rhGDF-5/β-TCP/PLGA group showed 2.4 times greater bone formation (height) (2.92 ± 0.66 versus 1.21 ± 0.30 mm, $P = 0.02$) and 2.1 times greater cementum formation (2.34 ± 0.44 versus 1.13 ± 0.25 mm, $P < 0.02$) than the control group (β-TCP/PLGA only). Increased PDL formation (1.57 ± 0.60 vs. 0.69 ± 0.61), bone area (0.84 ± 0.36 vs. 2.77 ± 1.38), and density (61.11 ± 5.33 vs. 68.92 ± 5.91) were also observed in the rhGDF-5/β-TCP/PLGA group compared with the control group; however, these differences did not attain statistical significance.

Kwon et al. (2010a)	BMP-14 GDF-5)	Dogs	Buccal dehiscence defects	PLGA construct	1. rhGDF-5/PLGA 2. Sham surgery	2, 4, 6 and 8 weeks	Cementum and PDL regeneration were twofold greater for the sham-surgery control compared with the rhGDF-5/PLGA construct at the 4-week observation interval (0.63 ± 0.07 vs. 1.31 ± 0.69 , respectively 0.56 ± 0.07 vs. 1.24 ± 0.68 , $P = 0.04$). In contrast, there was a significant increase in bone regeneration (height: 3.52 ± 0.33 vs. 2.03 ± 0.82 , $P = 0.002$; area: 2.62 ± 0.46 vs. 1.47 ± 0.76 , $P = 0.009$; and density: 60.74 ± 1.14 vs. 45.47 ± 10.78 , $P = 0.008$) at sites implanted with rhGDF-5/PLGA compared with control at this observation interval.
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ABS absorbable collagen sponge, PLGA poly(D,L-lactide-co-glycolide), Dex-GMA glycidyl methacrylated dextran, PGA-TMC polyglycolic acid-trimethylene carbonate, DFDBA demineralized freeze-dried bone allograft, hOP-1 human osteogenic protein-1, β -TCP β -tricalcium phosphate, β -NGF beta-nerve growth factor, PGA-TMC polyglycolic acid-trimethylene carbonate

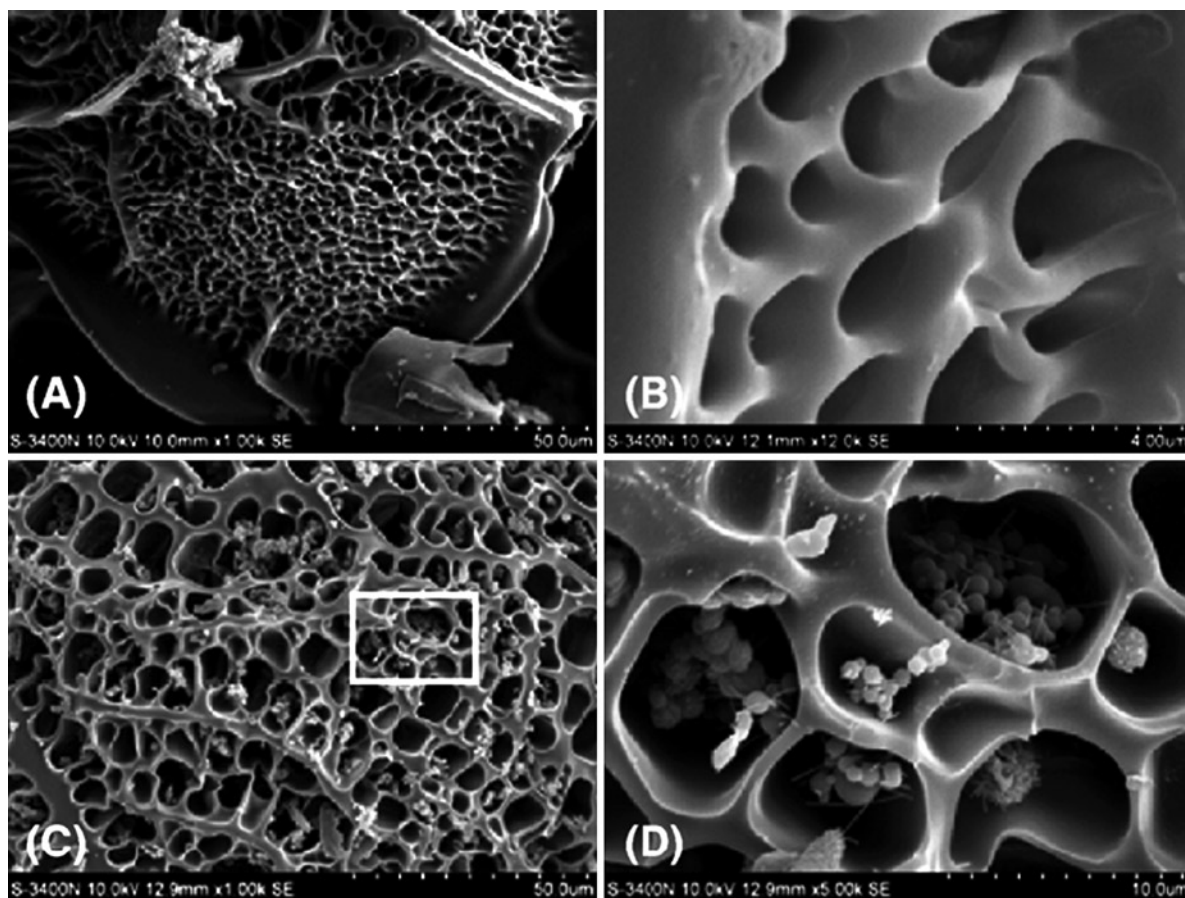


Fig. 5.26 SEM photographs (*cutaway view*) of glycidyl methacrylated dextran (Dex-GMA)/gelatin scaffolds obtained from 5 wt.% PEG, 10 wt.% Dex-GMA (DS = 6.3) and 5 wt.% gelatin in the polymerizing solution. The overall scaffold was clearly macroporous and presented an interconnected pore

structure (a) and (b), into which microspheres could be largely encased (c). (d) Magnified view of the *white rectangle* frame from (c). Magnification: $\times 1000$ (a), $\times 12,000$ (b), $\times 1000$ (c), $\times 5000$ (d) (Chen et al. 2007a. Reprinted with permission from Elsevier)

amounts to 1 $\mu\text{g/g}$ of fresh bone, making it difficult to obtain sufficient quantities to evaluate a therapeutic potential in clinically relevant skeletal defects (Urist et al. 1983; Huang et al. 2008). The concentration used in the animal models and humans are several orders of magnitude higher (Park 2009). In higher mammals, the BMPs may have to be present for a longer period to induce a local effect compared with small animals (Gautschi et al. 2007) and usually higher amount of BMPs are used. Dose-dependent effect on bone regeneration was evaluated in several studies, and higher concentrations in the study showed more effective for maxillary sinus floor augmentation procedures, as they enhanced bone formation and increased bone-to-implant contact (Park 2009; Boyne et al. 2005; Gruber et al. 2008; Groeneveld et al. 1999). Dose-related

responses to BMPs have also been observed in periodontal regeneration (Yan et al. 2010; Miyaji et al. 2010; Huang et al. 2005; Giannobile et al. 1998; Wikesjö et al. 2004). In contrast, several authors reported periodontal healing responses irrespective to the BMP concentration (Sorensen et al. 2004; Wikesjö et al. 1999; Ripamonti et al. 2002).

As Trombelli and Farina (2008) presented, the use of BMPs may lead to local adverse events, including root resorption (Sigurdsson et al. 1995; Wikesjö et al. 1999, 2003a; Selvig et al. 2002; Sorensen et al. 2004) and/or ankylosis (Sigurdsson et al. 1995, 1996; King et al. 1998b; King and Hughes 1999; Wikesjö et al. 1999, 2003a, b, c, 2004; Selvig et al. 2002; Saito et al. 2003; Sorensen et al. 2004; Takahashi et al. 2005; Kwon et al. 2010b; Miyaji et al. 2010). Ankylosis

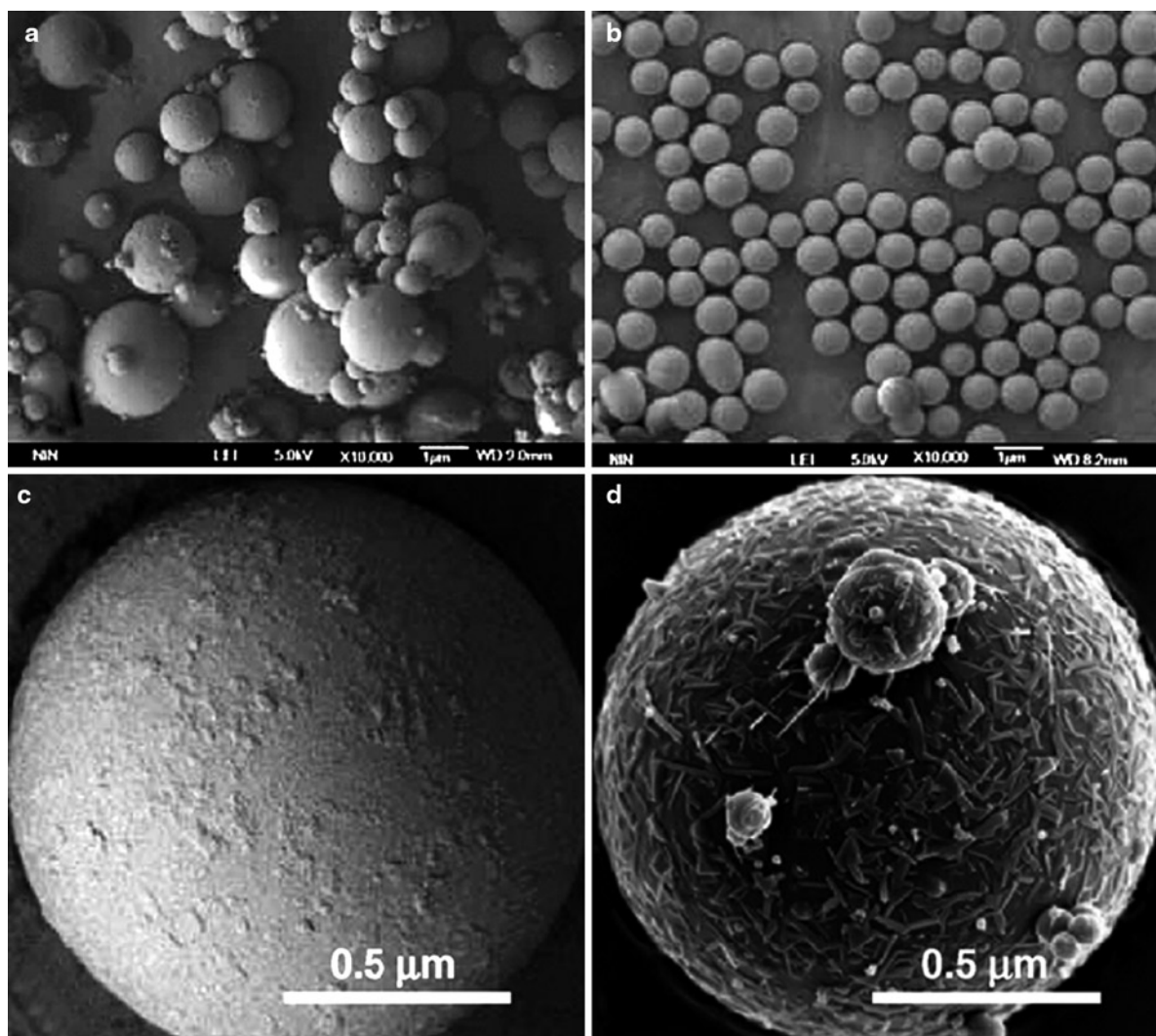


Fig. 5.27 SEM photographs of freeze-dried glycidyl methacrylated dextran/poly (ethylene glycol) (Dex-GMA/PEG) microspheres. (a) Microspheres before size selection with a particle size distribution ranging from 100 nm to 10 μ m. (b) Selected microspheres with a narrower size from 0.5 μ m to 1.5 μ m

for hydrogel-microsphere composition scaffold fabrication. Magnified view of BMP-free microspheres (c) and BMP-loaded microspheres (d). Magnification: $\times 10,000$ (a), $\times 10,000$ (b), $\times 50,000$ (c), $\times 50,000$ (d) (Chen et al. 2007a. Reprinted with permission from Elsevier)

appeared increased in sites receiving rhBMP-2/ACS compared to those receiving rhBMP-12/ACS (Wikesjö et al. 2004). Ankylosis was regularly observed located in the coronal third of the supraalveolar defect in the cervical third of the defects and was rarely encountered in the apical aspect of the supraalveolar periodontal defect (Kinoshita et al. 1997; Choi et al. 2002; Wikesjö et al. 2003a, 2004; Sorensen et al. 2004). Ankylosis do not appear to be a healing aberration in more limited periodontal defects or in the absence of extensive bone regeneration (Ripamonti et al. 1994;

Kinoshita et al. 1997; Choi et al. 2002; Wikesjö et al. 2004). Ankylosis was frequently observed at 10 days (the early stage of periodontal healing) but not at 38 days (the late stage) in rats have also been reported (King et al. 1997). Ankylosis disappearance following application of BMP has been observed not only in the case of a small defect in rats (King and Hughes 1999) but also in large periodontal defects such as horizontal defect and class III furcation defect in cats (Takahashi et al. 2005), which might be beneficial change for periodontal regeneration. King and Hughes (1999) reported

that the ankylosis disappeared after implantation of rhBMP-2 in experimentally created fenestration defects of rats. They found partial ankylosis at 10 days after implantation of rhBMP-2, but not at 35 days. Takahashi et al. (2005) revealed that ankylosis was observed after implantation of the BMP-2 in class III furcation defects in cats at 3 and 6 weeks, but not at 12 weeks. At 6 weeks after surgery, resolution of ankylosis with osteoclast-like cells was observed. Root resorption was observed around the area of ankylosis, and new cementum was formed on the root surface. A space resembling the periodontal ligament space was observed between new bone and the tooth root at an apical site of the area of ankylosis. At 12 weeks after surgery, ankylosis was not evident and almost complete regeneration of periodontal tissue was observed. The study also showed that the decomposition of PGS (polylactic acid–polyglycolic copolymer and gelatin sponge) combination had started during the 3 weeks after surgery, which resulted in the ankylosis formation. For the prevention of BMP-2-induced ankylosis, development of a carrier with the characteristics of slow decomposition was suggested (Takahashi et al. 2005). Root resorption that has also been reported in the above mentioned studies appears to be related to the concentration of rhBMP (Danesh-Meyer 2000).

Human trials evaluating the adjunctive clinical effect of BMP-2 and BMP-7 in the treatment of chronic periodontitis lesions are at present available (Bowers et al. 1991; Stavropoulos et al. 2009; Windisch et al. 2009; Sculean et al. 2009).

The evaluation of the effect of a combination of BMPs with a graft/bone substitute relates to one single histomorphometric study in humans where the association of BMP-3 (osteogenin) and two different biomaterials (purified bovine collagen and DFDBA) has been evaluated (Bowers et al. 1991). Test treatments consisted of the association of BMP-3 with DFDBA or bovine collagen; control groups consisted of the grafts used alone. Histologic evaluation at 6 months indicated that osteogenin combined with DFDBA significantly enhanced regeneration of a new attachment apparatus and component tissues in a submerged environment. DFDBA plus osteogenin and DFDBA alone formed significantly more new attachment apparatus and component tissues than either the tendon-derived matrix plus osteogenin or the tendon-derived matrix alone in both submerged and nonsubmerged environments. There

were no significant differences between the tendon-derived matrix plus osteogenin and the tendon-derived matrix alone in either the submerged or nonsubmerged environment (Bowers et al. 1991).

A Phase IIa randomized, controlled, clinical and histological pilot study was designed to evaluate rhGDF-5/TCP for periodontal wound healing/regeneration. Twenty chronic periodontitis patients, each exhibiting at least one deep intrabony defect with a probing depth ≥ 6 mm and an intrabony component ≥ 4 mm, had received basic periodontal therapy and exhibited high oral hygiene standards, were included in the study. One site/patient received rhGDF-5/TCP or served as sham-surgery control following stratified randomization. The postsurgery protocol included administration of augmentin (3×500 mg/day; 1 week), 0.2% chlorhexidine rinses (BID; 4 weeks) and professional supragingival cleanings every 14 days for 6 weeks and thereafter monthly. Block biopsies of the defect sites were collected at 6 months for histologic analysis. Mean ($\pm$ SD) defect depth was 5.1 ± 1.2 and 4.2 ± 1.9 mm for the rhGDF-5/ β -TCP and control defects, respectively ($P=0.39$). The mean bone regeneration height, was almost three times greater for the rhGDF-5/ β -TCP treatment compared with control (2.2 ± 1.6 versus 0.8 ± 1.0 mm; $P=0.08$). Similarly, PDL regeneration averaged 2.2 ± 1.4 versus 1.2 ± 1.0 mm ($P=0.26$), cementum regeneration 2.2 ± 1.4 versus 1.2 ± 1.1 mm ($P=0.26$) and bone regeneration area 0.7 ± 0.7 versus 0.3 ± 0.5 mm² ($P=0.14$). Residual β -TCP amounted to $8.4 \pm 11.5\%$. Ankylosis and root resorption were not observed (Stavropoulos et al. 2009). Treatment with rhGDF-5/ β -TCP resulted in greater PD reduction (3.7 ± 1.2 versus 3.1 ± 1.8 mm; $P=0.26$), less gingival recession (0.5 ± 0.8 versus 1.4 ± 1.0 mm; $P<0.05$) and almost two times greater CAL gain (3.2 ± 1.7 versus 1.7 ± 2.2 mm; $P=0.14$) at the deepest aspect of the defect compared to control (Windisch et al. 2009).

Related to data on patient-centered outcomes, the use of rhGDF-5/TCP for periodontal wound healing/regeneration was associated with minor to moderate adverse events, included postoperative swelling, headache and back pain (Sculean et al. 2009). The use of rhGDF-5/TCP (Sculean et al. 2009) and BMP-3 (osteogenin) (Bowers et al. 1991) appears safe and may substantially support periodontal wound healing/regeneration.

The optimal effects of BMPs are modulated by a range of factors that need careful evaluation in clinical

studies. These factors include the influence of root conditioning, occlusal loading, BMP dose and the release characteristics of the carrier as well as the suitability of the model to evaluate the efficacy of BMPs. Each of these factors may affect the rate of BMP-induced osteogenesis and cementogenesis and subsequent periodontal ligament (PDL) formation during the early and late stages of periodontal wound healing (King and Cochran 2002). The results suggested that the submerged defect model, where the root, periodontal defects and crown are under the mucoperiosteal flap (Sigurdsson et al. 1995; Bowers et al. 1991; Wikesjö et al. 1999, 2003a, b, c, 2004; Sorensen et al. 2004; Kwon et al. 2010b) has greater regenerative potential compared with the non-submerged defects despite the application of higher doses of rhBMP-2 in the non-submerged model. In non-submerged periodontal defects, the efficacy of BMPs may be modulated by the bacterial inflammatory component within the gingival crevice where proteases may degrade or interfere with the action of BMP. The formation of a long junctional epithelium may prevent BMP-stimulated PDL cells from migrating into the wound. Limited vascular supply from the mucosal tissue flaps may also account for the reduced efficacy of BMPs in non-submerged defects (King and Cochran 2002). It has been reported that root conditioning with 35% phosphoric acid gel impairs early BMP-induced osteogenesis while simultaneously promoting BMP-induced cementogenesis in rats (King et al. 1998b), while the demineralization of denuded root dentin surfaces with EDTA but stimulated cementum-like tissue formation and inhibited epithelial downgrowth in dogs (Miyaji et al. 2010).

It was also showed that the width of residual bone was one of the clinical host factors that affected bone regeneration following BMP implantation. However, it did not affect connective tissue attachment, cementum regeneration and downgrowth of junctional epithelium. The above result may be explained as follows. First, the wider the bone, the larger the reservoir of target cells originating from bone adjacent to the BMP/carrier, where rhBMP-2 induces osteoblastic differentiation and proliferation, in general. Second, the supply of preprogenitor mesenchymal cells, additional growth factor and nutrients from bone marrow increases because large areas of residual bone include more cancellous cellular marrow (Saito et al. 2009). Enhancing our understanding of other

clinical host factors, such as width of flaps, occlusion, density of blood vessels and inflammation, might improve the application of new bone formation in periodontal therapy (Saito et al. 2009).

Although BMP are the most effective osteoinductive materials known, there are problems associated with their use. BMP are produced by bone cells and stored in the extracellular matrix of bone at low levels and always in the presence of one or more inhibitors. This ensures that they are available when needed and that they are active only under specific conditions. When activated, the clearance rate for BMP is rapid. Thus, it is necessary to implant large concentrations of these proteins to have active protein present at the time when an appropriate responding cell population also is present. Moreover, BMPs are most effective when used with a carrier to retain the proteins at the implant site and to provide an osteoconductive matrix (Boyan et al. 2006). Some limitations of the approach to use of bone morphogenetic proteins (BMPs) in conjunction with a scaffold, are the large amount of protein required, the short protein half-life and poor retention of the protein in the defect site may be circumvented using gene therapy to trigger continued localized presence of an endogenously produced protein (Pierce and Mustoe 1995; Schek et al. 2006).

In the USA, currently only two growth factors (BMP-2 and PDGF-BB) have been approved by the FDA for selective clinical procedures in periodontology, although, several have been studied either alone or in combination (Elangovan et al. 2009). The combination of rhBMP-2 delivered in an absorbable type I collagen sponge was approved by the FDA in 2004 as INFUSE® bone graft (Wyeth Pharmaceuticals, Philadelphia, Pennsylvania) for anterior lumbar interbody spine fusion and open tibial shaft fractures and by the European Union (EU) in 2002 as InductOs® (Wyeth Pharmaceuticals, Maidenhead, Berkshire, UK) for open tibial shaft fracture. The product is also applied for bone augmentation for sinus lifting and implant dentistry (Allegrini et al. 2004; Schliephake et al. 2005; Ishikawa et al. 2009). At present, osteogenic protein-1 (OP-1) delivered in a particulate bone-derived type I collagen matrix is available in the USA and the EU as OP-1® implant (Stryker Biotech, Hopkinton, MA) through a Humanitarian Device Exemption (HDE) for recalcitrant nonunion fractures (Chen et al. 2009).

5.9 Growth Factor Combinations

Combinations of growth factors might be used to synergistically improve periodontal wound healing/regeneration. Most investigators have evaluated the effects of single factors only and might thus have overlooked potential large biologic responses comparable with those documented in the literature when growth factors used in combinations interact synergistically *in vitro* (Lee et al. 2010a).

The combination of several growth factors (platelet-derived growth factor-BB, insulin-like growth factor-1 and transforming growth factor- β 1), stimulated a mitogenic response and favored the adhesion of periodontal ligament cells *in vitro*, suggesting its possible role in periodontal regeneration (Sant'Ana et al. 2007). The *in vitro* presence of rhBMP-7, demineralized freeze-dried bone allograft (DFDBA), an inorganic bovine material with a synthetic peptide (PepGen P-15), rhBMP-7+DFDBA and rhBMP-7+PepGen P-15 promoted on human periodontal ligament (hPDL) cell a significant increase of alkaline phosphatase activity (Dereka et al. 2009). Zhang et al. (2009) demonstrated *in vitro* the promising potential of biomaterial expression of combinations of growth factors such as BMP-7 and PDGF-B for bone regeneration in tissue engineering applications. The combination of enamel matrix derivative and platelet-derived growth factor-BB (PDGF-BB) or the combination of enamel matrix derivative and insulin-like growth factor-1 were able to produce greater proliferative and wound-fill effects on periodontal ligament fibroblasts than each by themselves. If these combined effects can be translated clinically, one may see greater regeneration in periodontal defects with this combination (Chong et al. 2006; Palioto et al. 2004). The combination of PDGF-BB/ and insulin-like growth factor-1 (IGF-1) did not significantly improve the adhesion of cells compared to PDGF-BB alone, but did significantly improve adherence of human periodontal ligament fibroblast to tetracycline HCl conditioned periodontally involved root surfaces when compared to IGF-1 alone (Gamal et al. 1998). It was also suggested that, while rhPDGF-BB and IGF-1 stimulate proliferation and chemotaxis of PDL fibroblastic cells, the combination of these growth factors further increases the mitogenic effect (Matsuda et al. 1992).

Positive results in two animal models, the dog with natural periodontal disease and the nonhuman primate with ligature-induced attachment loss, demonstrated a

high degree of consistency in the effects of PDGF-BB/IGF-1 combination in promoting periodontal regeneration (Giannobile et al. 1994, 1996). At 1 month, PDGF-BB/IGF-1 administration resulted in a 64.1% and 51.4% increase in new attachment formation in the nonhuman primate and canine, respectively, while controls (surgery plus placebo) demonstrated 34.1% and 8.6% increases in new attachment formation in the nonhuman primate and canine models, respectively. Further, application of PDGF-BB/IGF-1 stimulated 21.6% and 65% osseous defect fill in the nonhuman primate and canine, respectively, while controls demonstrated 8.5% and 14.5% osseous defect fill in the nonhuman primate and canine, respectively (Giannobile et al. 1994). In a ligature-induced periodontitis in nonhuman primates, it was demonstrated that IGF-1 alone at the dose tested did not significantly alter periodontal wound healing, PDGF-BB alone significantly stimulated new attachment, with trends of effect on other parameters, while the PDGF-BB/IGF-1 combination resulted in significant increases in new attachment and osseous defect fill (Giannobile et al. 1996). The PDGF-BB/IGF-1 combination applied to periodontitis-affected teeth in dogs following open flap debridement induced significant amounts of new bone and cementum formation. A nearly continuous layer of osteoblasts lined the newly formed bone, and there was a dense cellular "front" at the coronal extent of the new bone, while histological analyses of control specimens revealed a long junctional epithelial attachment, and no new bone or cementum formation (Lynch et al. 1989c). In a similar study, the same growth factors combination induced, at 2 and 5 weeks postoperatively, a significant five- to tenfold increase in new bone and cementum compared to controls receiving the placebo gel. The new bone underwent a normal maturation process as judged by histologic appearance. A physiologic periodontal ligament space was also formed between the new bone and new cementum. There was no increase in ankylosis in the treated sites.

The safety of rh PDGF-BB and rhIGF-1 was assessed also assessed in humans. Thirty-eight human subjects possessing bilateral osseous periodontal lesions were assigned to one of two treatment groups in a split-mouth design. Two dose levels were tested, 50 μ m/mL each of rhPDGF-BB and rhIGF-1 in a gel vehicle (LD-PDGF/IGF-1) and 150 μ m/mL each of rhPDGF-BB and rhIGF-1 plus vehicle (HD-PDGF/IGF-1). Control treatment

consisted of either conventional periodontal flap surgery or surgery plus vehicle. No local or systemic safety issues were found as a result of growth factors administration. No patients developed antibodies to the growth factors proteins. LD-PDGF/IGF-I did not elicit increased defect fill compared to the control; however, HD-PDGF/IGF-I resulted in a significant promotion in bone regeneration: a statistically significant increases in alveolar bone formation were noted as measured by surgical re-entry 9 months following drug delivery ($P < 0.05$). This corresponded to an increase of 2.08 mm of new vertical bone height and 42.3% osseous defect fill in the HD-PDGF/IGF-I subjects versus only 0.75 mm and 18.5% gains in new bone height and osseous fill, respectively, in the controls. Furcation lesions, although limited in number, responded most favorably to treatment, with 2.8 mm horizontal osseous fill. The results from this study suggested that the local application of rhPDGF-BB and rhIGF-1 to periodontal lesions is safe at the dose levels studied (Howell et al. 1997).

However, despite these preliminary results who suggested that *in vivo* application of the combination of PDGF and IGF-1 may enhance regeneration of the periodontal structures, it was hypothesized that growth factor combinations for periodontal wound healing/regeneration may never be developed for clinical use because of the substantial, complex and costly evaluation needed to meet regulatory demands (Lee et al. 2010a).

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Index

A

Acellular dermal matrix (ADM), 18, 43–45
 Aligipore®, 102, 110
 Alloderm, 43
 Allografts, 24, 29, 43–45, 47, 73, 77–85, 87, 96, 118, 120, 123, 124, 145, 174, 186, 188, 228, 230, 243, 246, 249, 287, 292
 Allomatrix®, 115
 Alloplastic synthetic grafts, 94–123
 Alloplasts, 94–123
 American Association of Tissue Banks (AATB), 79, 85
 Amniotic membrane (AM), 44, 45
 Angle of the defect, 194
 Anorganic bovine-derived bone xenograft (BDX), 86–90, 167, 170, 179, 183, 185
 Antibiotics, 22–23, 52, 94, 107, 127, 234
 Atrisob barrier®, 48
 Atrisorb, 31, 50, 54
 Atrisorb®-D FreeFlow™, 50, 54
 Atrophied alveolar ridges, 87
 Autogenous bone grafts, 73, 74, 76–78, 116, 246
 extraoral, 75–77
 intraoral, 74–75
 Autografts, 74–77, 123, 124

B

β-tricalcium phosphate, 102, 103, 107–114, 245, 246, 259, 287
 Bicon, 40, 109
 Bioactive glasses (BG), 78, 109, 116–121, 169, 170, 172, 179, 180, 188, 234
 Biobar, 40
 Biocoral (Inotek, Saint Gonnery, France), 90, 91
 Bio-Gide®, 31, 32, 34, 37–43, 100, 189
 Bioglass, 116, 117, 119, 120, 268
 Biogran™, 121
 Biomaterial, 18, 31–33, 35–37, 44, 75, 84, 86, 90, 93, 96, 101, 102, 105, 112, 120, 173–177, 179, 228, 230–234, 238, 250, 262, 265, 268, 286, 290, 292
 BioMend™, 25, 32, 34, 37, 40–42
 BioMend Extend™, 40–42
 Bio-Oss®, 38, 41, 42, 86–91, 102, 123, 184, 228, 268, 270
 Bio-Oss Collagen®, 87–89
 Bioresorb®, 109
 Biostite®, 40, 102
 Biphasic alloplastic materials, 102–103, 109

Blend of cortical and cancellous intraoral bone, 75
 Bone Ceramic®, 103, 109, 110, 188
 Bone filters, 75
 Bone morphogenetic proteins (BMPs), 81, 82, 84, 86, 95, 124, 145, 148, 208, 227, 229, 231, 232, 234, 237, 260, 262, 292
 Bone replacement grafts, 9, 11, 13, 73, 82, 85, 87, 126, 127

C

Calcitec® Inc., 103, 109
 Calcitite, 96
 Calcium hydroxide (CaOH₂), 94, 121–123
 Calcium phosphate cement (CPC), 103–107, 234, 268, 275
 Calcium phosphates, 112, 116
 Calcium sulfate, 24, 85, 112, 114–116, 120, 121, 228, 230
 Calforma™ calcium sulfate bone graft barrier, 115
 Calmatrix, 115
 Capset® calcium sulfate bone graft barrier, 115
 Cargile membranes, 43
 Cerabone®, 98, 100, 110
 Ceraform®, 103, 110
 Cerasorb®, 109, 110, 188, 189
 Ceros®, 109
 Chitosan (CS), 106, 230–232, 234
 Chlorhexidine, 16, 24, 37, 55, 75, 127, 192, 290
 Chronos®, 109
 Citric acid, 6, 33, 55, 83, 217–222
 Colética, 31
 Collacote®, 230, 282
 Collagen, 3, 76, 145, 217, 228
 Collagenase, 36, 41
 Collagen membranes, 4, 6, 26, 36–41, 43, 51, 52, 54, 55, 87, 92, 100, 116, 273, 274
 Collagraft®, 112, 124
 Collaplug®, 230
 Composite graft, 88, 115, 123–125
 Coral graft substitutes, 92
 Coralline calcium carbonate, 78, 92–94
 Cortical bone chips, 74
 Cross-linking techniques, 37

D

Demineralized dentin matrix (DDM), 95
 Demineralized freeze-dried bone allograft (DFDBA), 77, 79–85, 96, 115, 116, 118, 119, 184, 186, 188, 243, 249, 287, 290, 292

Dense polytetrafluoroethylene (dense-PTFE), 27
 Diphenylphosphoryl azide (DPPA), 36, 40, 43
 Doxycycline, 37, 50, 54, 55, 220
 Dura mater, 24, 43

E

Enamel matrix derivative (EMD), 6, 17, 19, 75, 81, 82, 87, 109, 112, 118–120, 145–209, 222, 223, 228, 229, 259, 260, 292
 Epi-Guide®, 25, 50, 51
 Ethylenediaminetetraacetic acid (EDTA), 150–152, 156, 157, 159, 162–165, 168, 195, 196, 217–223, 281, 291
 Expanded polytetrafluoroethylene (ePTFE), 4, 7, 8, 12–14, 23–31, 38, 39, 50, 54, 82, 116, 118, 276, 277
 Experimental Mempo®, 50

F

Fibroblast growth factors (FGFs), 82, 114, 148, 208, 227, 229, 237, 250–254, 257, 258, 260
 Fluorohydroxyapatite (FHA), 101, 102
 Fortoss® vital, 112, 115, 116
 Freeze-dried bone allografts (FDBA), 29, 43, 47, 75, 77, 79–81, 85, 96, 117, 145, 188, 243, 245, 246, 249, 287, 292
 Freeze-dried fascia lata, 24, 31
 FRIOS®, 99, 102
 Furcation, 3, 76, 147, 221, 241

G

Gelfoam®, 230
 Gingival recession, 3–5, 14–23, 27, 29, 45, 48, 50, 96, 109, 118, 155, 161, 171, 174, 177, 179, 183, 186, 187, 194, 196–209, 222, 246–248, 290
 Gore-Tex (GT), 25, 28, 29, 46, 50, 54, 181, 182
 Growth factor combinations, 292–293
 Growth factor-enhanced matrix (GEM-21S®), 246
 Growth factors (GFs), 27, 73, 146, 227
 Guidor®, 25, 46, 47, 50, 52
 Gypsum, 114

H

Healos®, 112, 124
 Helistat®, 230, 268
 Hyaluronic acid (HA), 56, 57, 230
 Hydroxyapatite, 40, 52, 56, 79, 86, 89, 92, 95–104, 107, 109, 110, 112, 116, 123, 221, 234, 246, 249, 268
 Hydroxyapatite nanocrystalline, 98–101
 Hydroxylapatite (HA), 89, 93–103, 188
 coralline porous non-resorbable, 98
 pure densely sintered, 96–98
 resorbable non-ceramic, 98

I

Implants, 9, 24, 43, 44, 48, 50, 81, 87, 90, 95, 103, 104, 121–123, 232, 240
 Infrabony defects, 1–9, 85, 120, 146, 173, 175, 176, 178, 192–195, 209, 245, 247
 Insulin-like growth factors (IGFs), 84, 227, 229, 231, 237, 244, 245, 249–250, 292
 Interpore 200, 92, 98
 Intrabony defects, 1, 75, 153, 222, 243

L

Lambone, 31
 Laminar bone barrier, 31

M

MBCP, 103, 109
 Membrane exposure, 7, 16–18, 23, 24, 28, 38, 158, 209
 Membrane non-absorbable, 20–21, 25–31
 Meta-analysis, 4, 6, 11, 12, 14, 17–19, 22, 44, 82, 93, 94, 96, 173–180, 183, 190–193, 198, 205, 218, 221, 222
 Metronidazole, 37, 55, 56, 195
 Millipore filter, 25, 27
 Monocalcium phosphate anhydrous, 103

N

Nano-carbonated hydroxyapatite, 52

O

Octacalcium phosphate, 103, 104
 Oily CaOH₂ suspension, 121, 123
 Open flap debridement (OFD), 3–4, 6, 11, 12, 73, 74, 7678, 82, 9296, 101, 104, 109, 112, 113, 156, 171–179, 192, 195, 196, 222, 223, 247–249, 259, 292
 Opocrin, 40, 42, 43
 Osseoguard™, 40, 42, 43
 Ossequest™, 46, 48
 Osseous coagulum, 75
 Ossix™, 34, 37, 43, 56, 57
 Ossix plus, 40
 Osteoconduction, 73, 81, 117, 239
 Osteogen®, 98, 103, 109
 Osteograf/D300, 98
 Osteograf/D700, 98
 Osteograf/LD-300, 98
 Osteograf/N®, 87, 89
 Osteoinductal®, 121, 123
 Osteoinduction, 73, 81, 82, 84
 Osteon™, 92, 98, 109, 155
 Osteoset®, 115
 Ostim™, 98, 101
 Oxidized cellulose mesh, 43

P

Paroguide, 40, 43
 Patient's compliance, 8, 15, 52, 126
 Pentadecapeptide (P-15), 90, 246
 Pepgen P-15®, 87, 90, 91, 246, 292
 Periapical lesions, 87
 Peri-implantitis defects, 87
 Periodontal regeneration, 1, 24–58, 73, 145, 217, 227–293
 Periodontal surgery, 29, 74, 126, 147, 194, 195, 197, 209, 217, 222, 241, 260
 Periogen, 40
 Perioglas®, 86, 120, 121, 179, 185, 187–189
 Perioglas® Plus, 120, 121
 Phosphoric acid, 217, 272, 273, 291
 Piezoelectric device, 75
 Piezosurgery, 75
 Plaster of Paris, 114, 115
 Platelet-derived growth factor (PDGF), 82, 84, 114, 118, 227, 229, 237, 243–245, 250, 259, 260, 262

Platelet-derived growth factor-BB (PDGF-BB), 56, 84, 107, 244, 246, 291, 292
Platelet-rich plasma (PRP), 56, 58, 81, 84, 85, 87, 118, 227, 228, 235–243, 249, 260, 262
PMMA-PHEMA polymers, 79, 94, 95
Poly(DL-lactic acid) (PDLA), 55
Poly(DL-lactide-co-glycolide) (PDLGA), 55
Polyglactin 910, 31, 48, 50, 52
Polyglycolic acid, 24, 26, 31, 45, 46, 56, 124, 228, 230, 287
Polyhydroxybutyrate (PHB), 24, 31
Polylactic acid, 24, 26, 31, 38, 40, 46, 50, 51, 55, 79, 268, 270
Polymers, 33, 45, 46, 50, 52, 9495, 104, 228, 230, 232–234, 268
Polytetrafluoroethylene (PTFE), 7, 8, 11, 24, 25, 27–30, 32, 38, 39, 82, 230
Polyurethane, 24, 52
Porites, 92, 98
Pro-Osteon 500R, 98
Pulp capping, 121

R

Recession gingival *See* Gingival recession
Residual bony walls, 2, 6, 126
Resin–ionomer membrane, 27
Resolut Adapt® LT, 46
Resolute™, 46
Resolut XT™, 31, 46
Root conditioning, 6, 11, 13, 17, 18, 21, 196, 217, 220–223, 272, 273, 291
Root exposure, 14
Root sensitivity, 14
Rubber dam, 27

S

Silica gel, 116
Sinus elevation floor procedures, 87
Sinus lift procedures, 121
Smoking, 7, 8, 15, 22, 118, 126, 162, 180, 192, 194,
Suprabony defects, 1, 154, 197
Suprabony lesions, 197
Synthograft™, 109

T

TefGen, 28, 32
Tetracalcium phosphate, 103, 104, 106
Tetracycline-coated ePTFE membranes, 54
Tetracycline hydrochloride, 217, 220
Tetracycline-loaded poly(L-lactide) membranes, 54
TissueGuide, 40
Titanium-reinforced ePTFE, 29–31
Tobacco, 126
Tooth avulsion, 208
Tooth replantation, 208
Transforming growth factor-beta (TGF-beta), 82, 229, 260, 262, 265
Tricalcium phosphate (TCP), 102, 103, 106116, 188, 230, 242, 244–246, 254, 259, 287, 290
Tricos®, 103, 109, 112, 124
Tutodent, 32, 34, 37, 40
Tutoplast®, 110

U

Unigraft®, 121

V

Vicryl, 31, 48, 50, 172, 189
Vicryl periodontal mesh™, 48
Vitoss®, 109

W

Wound healing, 6, 18, 33, 42, 44, 73, 87, 122, 123, 145, 208, 233, 243, 244, 246, 254, 255, 260, 273, 274, 290–293

X

Xenografts, 29, 73, 86, 92, 100, 102, 125, 169, 170, 179

Z

Zeta potential, 112